

From genomic complexity to diagnostic value

NGS, PCR and integrated workflows
in advanced molecular diagnostics

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Executive Overview

Why this White Paper?

Molecular diagnostics is entering a phase in which PCR and NGS can no longer be viewed as isolated technologies. Technology choice increasingly depends on the question: known targets, routine testing and targeted measurements in some cases; genomic breadth, custom panels, bioinformatics and the interpretation of complexity in others. This White Paper explains how Helyx Industries S.p.A. sees this evolution: as the development of an ecosystem in which different capabilities meet different needs.

The document focuses on the role of NGS as molecular diagnostics matures, placing it alongside clinical PCR and distributed PCR. Mytho represents the Group's custom NGS capability; Vytro maintains the connection with clinical PCR, defined targets and the laboratory; Hyris brings PCR into distributed and multisite models. The central argument is simple: value arises when technology is selected, designed and managed for its intended context of use.

Scope of analysis.

The White Paper follows a progressive path. It begins with the maturation of molecular biology, examining how PCR moved from a scientific breakthrough to a routine technology, and how NGS is becoming more accessible while the complete workflow remains complex. It then explores the Helyx structure and the roles of its three divisions, with particular attention to Mytho as a custom NGS capability built on tailored panels, bioinformatics, data quality and specialist workflows.

The document explains how PCR and NGS complement each other through the principle of diagnostic appropriateness. PCR remains central for known targets, routine testing, targeted measurements and follow-up. NGS becomes relevant when the question involves many genes or variants, biological heterogeneity or extended characterisation. Two applications make this approach concrete: the public Genoma-Puglia initiative, an example of NGS in a complex institutional programme, and microbiota analysis, a model of an integrated workflow combining broad profiling with targeted measurement.

Who it is for.

This White Paper is intended for molecular biology and genetics laboratories, hospitals, clinical stakeholders, industrial and scientific partners, institutional representatives and investors. For laboratories, it offers a framework for choosing between PCR and NGS according to the question at hand. For partners and clinical stakeholders, it explains where custom NGS can create value: panels, data quality, bioinformatics and workflow traceability. For institutional representatives and investors, it sets out Helyx's industrial direction at a time when applied genomics is maturing but still requires expertise, real-world cases and governance.

This document is neither a product catalogue nor a broad technological promise. It is a technical and industrial positioning paper that describes roles, adoption logic, applications and the conditions under which value is created.

How to read it.

Reading the paper from start to finish traces the full argument: from the maturation of PCR and NGS to the development of the Helyx ecosystem, from Mytho's contribution to diagnostic complementarity, and then to applications and the strategic outlook.

Readers interested in the technological rationale can begin with the opening chapters on the maturation of molecular biology and the value of NGS. Those seeking an operational perspective can focus on the section about diagnostic appropriateness in PCR and NGS. Readers interested in applications will find two different examples of managed complexity in the Genoma-Puglia programme and microbiota analysis: an institutional setting on one hand and an integrated workflow on the other. The appendices bring together Q&A, annotated sources and an essential glossary for quick reference without overloading the main text.

Introduction

This White Paper starts from a transition already visible in laboratories: different technologies are taking on more distinct and complementary roles. PCR has completed much of its path to maturity. From a scientific breakthrough, it has become a robust, repeatable technology, integrated into laboratories and central to many diagnostic workflows. NGS, or Next-Generation Sequencing, is at a different stage of a similar evolution: generating genomic data has become more accessible and clinical and institutional applications are expanding, while the complete process remains highly dependent on specialist expertise.

This evolution leads to the paper's central argument: the diagnostic question guides the choice of technology. When a laboratory needs to examine a few known targets, perform routine testing, monitor selected markers or obtain focused, repeatable answers, PCR retains a natural role. When the question involves many genes or variants, substantial biological heterogeneity, custom panels or a broader view of genomic complexity, NGS becomes the more suitable tool.

In many settings, PCR and NGS form part of the same pathway. Broad profiling can reveal signals that warrant further investigation. Targeted measurement can track selected targets. A workflow may call for an extended analysis at one stage and a more focused answer at another. The complementary use of the two technologies thus becomes an operational language for advanced molecular diagnostics.

Helyx Industries S.p.A. participates in this evolution through an industrial structure with distinct roles. Hyris brings PCR to distributed, multisite and operationally flexible models. Vytro structures clinical PCR in the laboratory, focusing on defined targets, IVD where applicable, deep multiplexing and fields such as molecular microbiology, infectious diseases, virology, genetics, oncohaematology and pharmacogenetics. Mytho represents the Group's custom NGS capability: tailored panels, advanced customisation, bioinformatics and specialist workflows.

This organisation reflects a situation already familiar to molecular biology and genetics laboratories. Often the stakeholder is the same; the breadth of the question changes. PCR is appropriate when the question concerns a few targets, variants or polymorphisms. NGS comes into play when the number of genes, regions or alterations to examine grows substantially. Value lies in choosing the technology best suited to the need, rather than favouring one platform in every case.

Mytho fits into this direction as a specialist capability for complex genomic questions. NGS generates substantial volumes of data, but data alone are insufficient. A useful NGS process requires panel design, sample quality, workflow control, a bioinformatics pipeline, annotation, classification, reporting and an output aligned with the question posed by the laboratory, geneticist or partner. Mytho occupies this space: custom NGS expertise for projects in which genomic depth must become usable information.

The applications discussed in this paper show two complementary directions. The public Genoma-Puglia initiative, within the industrial collaboration between Revvity Italia and Helyx, shows how NGS can become part of complex institutional programmes. Genomic newborn screening requires selection criteria, data quality, governance, diagnostic confirmation and integration into clinical pathways. Its value lies in making sequencing part of a documented and well-governed programme.

Microbiota analysis illustrates a second direction. A complex microbial community may require broad profiling to observe composition, diversity, patterns and biological signals. At this stage, NGS and metagenomics allow extended analysis. When selected targets or more targeted measurement needs emerge, PCR can complement profiling with a more focused level of analysis, always according to the setting, validation and intended use.

The strategic moment for NGS is now: the technology is mature enough to extend beyond research, yet complex enough to require expertise, method and credibility. The falling cost of data generation has made sequencing more accessible, but the complete process still depends on bioinformatics, quality, interpretation, reporting and governance. At this stage, building applications, workflows, partner relationships and custom capabilities means preparing for the continued maturation of applied genomics.

Helyx sees NGS as an expansion of its diagnostic ecosystem. Mytho adds genomic depth and custom capability; Vytro maintains the connection with clinical PCR, defined targets and the laboratory; Hyris brings PCR into distributed models. The strength of the approach lies in matching different technologies to different questions while maintaining clear roles, workflow quality and consistency in application.

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Chapter 1

From PCR to NGS: when a technology becomes diagnostic

In molecular diagnostics, a discovery becomes truly significant when it can be turned into a process that others can adopt. This requires properly managed samples, repeatable methods, quality controls, trained staff, available instruments, stable reagents and interpretable results. Analytical power opens a possibility; operational maturity makes it applicable in the laboratory, in clinical practice or in industrial settings, according to intended use, validation and governance.

PCR is one of the clearest precedents for this path. It introduced a new technical ability: amplifying specific DNA sequences from very small amounts of genetic material. Instruments, protocols, expertise and applications gradually developed around that ability. NGS is now at a different stage of a similar evolution: it provides much richer information and data generation is increasingly accessible, but it remains demanding in terms of workflow, bioinformatics and interpretation.

Molecular diagnostics advances when the question guides the choice of technology. Speed and a known target call for one answer; genomic depth and breadth of information call for another. In some pathways, the technologies are combined. PCR and NGS form part of the same evolution in molecular biology, with distinct and complementary roles.

1.1 PCR: when amplification changes the laboratory

The Polymerase Chain Reaction profoundly changed how molecular biology examines DNA. In 1993, Kary B. Mullis received the Nobel Prize in Chemistry for inventing PCR. The method can produce large amounts of DNA from a very small starting quantity, enabling important applications in medical research, molecular diagnostics and forensic science.

The value of PCR also lies in how testing is organised. The ability to amplify a specific sequence makes it possible to examine a molecular target precisely, even when little material is available. This has supported applications in research, genetics, molecular microbiology, oncology, biological quality control and surveillance.

PCR spread through a combination of factors: more accessible instruments, established protocols, standardised reagents, quality control, staff training and integration into laboratories. An effective reaction becomes a routine technology when the entire process, from sample to result, can be repeated, controlled and interpreted.

This lesson still applies. Every new molecular technology passes through a stage in which scientific performance must be matched by operational discipline, economic sustainability and confidence in the result.

1.2 Diagnostic maturity: the conditions for adoption

A mature molecular technology has recognisable features. It must be repeatable, so results remain consistent over time and across operators and samples. It must be standardised, so protocols, controls and instruments reduce variability. It must be operationally accessible, so costs, skills and infrastructure fit the intended setting. It must be interpretable, so the output can inform a decision. It must be governed, because quality, traceability, accountability and error management are part of diagnostic value.

Testing for SARS-CoV-2 made this infrastructure visible beyond specialist laboratories. Nucleic acid amplification tests, including PCR and other NAAT technologies, entered the language of public health, policy documents and health communication. The pandemic clearly showed that a molecular test creates value when it is integrated into a system: sample collection, workflow, turnaround time, result quality and decision-making capacity.

PCR reached this stage after decades of maturation. NGS is now moving along a more recent curve of accessibility and adoption, with different levels of complexity and a greater dependence on workflows, bioinformatics and interpretation.

1.3 NGS: increasing accessibility and manageable complexity

Next-Generation Sequencing opens up a different level of information. NGS technologies can read many genomic regions, extensive panels, exomes or whole genomes at the same time. This matters when the question requires breadth: rare variants, multigene profiles, biological heterogeneity or a richer analysis than a single target can provide.

One factor making NGS more accessible is the falling cost of generating sequencing data. Historical data from the National Human Genome Research Institute show a marked decline in sequencing costs, with a clear change in trajectory following the introduction of second-generation technologies.

This accessibility must be understood precisely. The cost of producing data is not the cost of the complete NGS process. Sample quality, library preparation, the sequencing run, quality control, the bioinformatics pipeline, annotation, interpretation, reporting and data governance remain central.

Data generation has become much more efficient, but adoption in diagnostic and applied settings still requires expertise, workflows and control systems. The value of NGS lies in turning an information-rich technology into a repeatable, traceable and useful process.

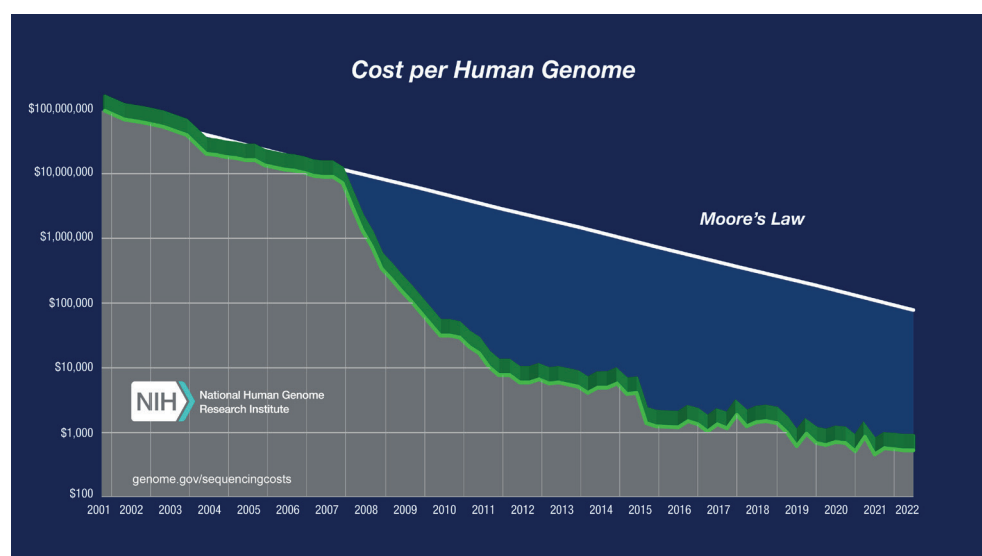
Sequencing cost per human genome

The decline concerns the cost of generating sequencing data, not the complete NGS process. Downstream activities such as bioinformatics, variant identification, interpretation and reporting are excluded.

Source

NHGRI, DNA Sequencing Costs: Data, "Cost per Human Genome". Courtesy: National Human Genome Research Institute / genome.gov.

Last updated: 16 May 2023.



1.4 From sequence to meaning

As data generation becomes more accessible, value shifts towards what happens after sequencing. Sequences, variants and biological signals must become usable information. In NGS, this means designing the panel correctly, generating high-quality data, applying validated pipelines, keeping databases current, interpreting complex results and producing outputs suited to the clinical, laboratory or research setting.

Precision oncology provides a clear example of this maturation. ESMO recommendations on the use of NGS in patients with advanced cancers place sequencing in specific clinical contexts, where genomic profiling can help with stratification, the identification of actionable alterations or access to targeted therapies.

The same principle appears in the validation of bioinformatics pipelines. AMP/CAP recommendations describe NGS pipelines as integral parts of the assay: not an optional step, but a part of the process that affects result quality, reproducibility and reliability.

NGS thus becomes an analytical and information pathway. The sequence starts the process; a useful result emerges from the interaction of laboratory work, bioinformatics, quality, interpretive rules and clinical responsibility.

1.5 The clinical question as a selection criterion

As molecular technologies mature, the clinical question plays a central role in selecting the method.

PCR is especially suitable when the target is known, a rapid answer is needed, the workflow requires high repeatability, and the laboratory must handle routine testing, monitoring or targeted confirmation. Its strength is the ability to deliver focused, efficient answers that fit standardised processes.

NGS is appropriate when the question requires breadth. Many genes, many variants, complex profiles, rare diseases, genomic screening, precision oncology and biological heterogeneity may make a target-by-target approach fragmented and limit the information available. NGS broadens the picture and can examine several levels within one analytical strategy, while placing greater demands on interpretation and governance.

Complementarity is evident in real workflows. One pathway may use NGS for in-depth profiling and PCR to follow specific targets over time. In other cases, PCR is the most efficient choice from the outset. Elsewhere, NGS can bring together analyses that would otherwise require many separate tests.

Helyx Industries S.p.A. interprets this evolution through complementary capabilities in advanced molecular diagnostics: distributed PCR, clinical PCR with deep multiplexing and custom NGS. This structure matches different technologies to different questions while maintaining clear roles and consistency in application.

Molecular diagnostics advances when laboratories, clinicians and technology partners choose the best combination for the problem: speed and routine testing when focus is needed; genomic depth and interpretability when breadth is needed; and technology integration when the workflow calls for it.

Chapter 2

The Helyx ecosystem:
three divisions, one integrated
diagnostic approach

2.1 An ecosystem built for different diagnostic questions

Advanced molecular diagnostics requires different technologies within different workflows. A test for a known target has different operational requirements from a custom NGS panel, a regulated clinical routine or a distributed PCR model. Value comes from selecting and managing the solution best suited to its intended setting, while keeping the technologies, markets, levels of evidence and regulatory responsibilities clear.

Helyx Industries S.p.A. organises this approach through three divisions with distinct roles. Hyris, Vytro and Mytho represent three complementary areas of molecular diagnostics: distributed PCR, clinical PCR with deep multiplexing and custom NGS. This structure places NGS within a broader system rather than viewing next-generation sequencing in isolation.

Mytho provides the Group's custom NGS capability; Vytro maintains the connection with molecular biology and genetics laboratories, clinical PCR, defined targets and deep multiplexing; Hyris extends PCR to distributed and multisite models. NGS is better understood when considered alongside mature molecular technologies and the clinical or operational workflows in which PCR continues to perform essential functions.

The Helyx structure is more than the sum of its divisions. It is designed to address different diagnostic questions: routine testing and known targets, targeted investigation, broad genomic analysis and distributed deployment. PCR and NGS complement each other because their roles are distinct.

HYRIS

Distributed PCR: platforms and services that bring genetic testing beyond the laboratory, with repeatable workflows and centralised data management.

VYTRO

Clinical IVD PCR: kits, panels and reagents for laboratories and hospitals, focused on standardisation, compliance and reliability.

MYTHO

Mytho — tailored NGS: panel design and bioinformatics that turn complex genomic data into usable insights.

When testing must be deployed across multiple sites or operational settings.

When the question concerns a few defined targets, variants or polymorphisms.

When the number of genes, regions or alterations to examine grows.

2.2 Hyris: distributed PCR and operational architecture

Hyris represents the Group's distributed PCR capability. Hyris System™ combines hardware, software and reagents to bring molecular testing into operational settings that need not be centralised.

Its role is to move molecular testing closer to the point of use while supporting controlled workflows, connectivity and traceable result management. This encompasses multisite deployment, distributed environments, repeatable processes and operating models in which logistics, speed and traceability affect the value of testing.

In advanced molecular diagnostics, Hyris shows that accessibility depends on more than analytical technology. It also depends on how a test is deployed in the right setting, managed over time and integrated into verifiable processes. The operational placement of a test therefore becomes part of its value.

Hyris remains focused on distributed PCR and Hyris System™. Its contribution is architectural and operational: it shows how molecular diagnostics can become more accessible, repeatable and manageable beyond traditional centralised models.

2.3 Vytro: clinical PCR, IVD and deep multiplexing

Vytro provides the clinical laboratory dimension of PCR for molecular biology and genetics laboratories, hospitals and diagnostic centres. The division develops PCR solutions for various molecular applications, including IVD products where applicable and in line with intended use, validation and relevant regulatory requirements.

Its catalogue covers molecular microbiology, infectious diseases and virology, as well as genetics, oncohaematology and pharmacogenetics. In the latter fields, the overlap with NGS is particularly marked. When the question concerns a few defined variants, polymorphisms or targets, PCR provides targeted investigation. As the number of variants, genes or regions to examine grows, NGS better meets the need for breadth.

This continuity also matters to users. A molecular biology laboratory and a geneticist may work in the same setting while choosing the technology best suited to the question: PCR for focused answers about known targets, NGS when genomic complexity requires more extensive analysis. In IVD settings, intended use, validation and performance evaluation remain central. References to diagnostic products must therefore be tied to the intended application, applicable requirements and available evidence, avoiding generic claims.

Vytro's deep multiplexing increases the information density of PCR across predefined targets. This proprietary technology aims to make PCR workflows richer and more targeted while retaining a target-driven approach. Specific applications remain linked to their respective intended uses and the data available for each setting.

In this view, Vytro represents the clinical laboratory layer of the more mature, regulated and focused part of the Helyx ecosystem. It connects naturally with Mytho when the same laboratory must choose between targeted PCR investigation and broader NGS analysis.

2.4 Mytho: custom NGS, bioinformatics and advanced customisation

Mytho represents the Group's custom NGS capability. Its expertise includes tailored NGS panels, advanced sequencing solutions and bioinformatics pipelines designed to support specialist clinical, research or partnership applications.

Mytho's value rests on a central principle of applied genomics: generating data does not automatically produce usable information. A result takes shape through panel design, data quality, pipeline robustness and the ability to deliver an output suited to its intended setting.

Mytho combines NGS and bioinformatics expertise to support custom panels and specialist workflows, with an approach focused on quality, traceability and project-specific design. Bioinformatics makes data easier to understand and manage; clinical interpretation remains dependent on the context, the available evidence and the responsibilities assigned within the pathway. Mytho thus brings genomic depth to the Helyx ecosystem: tailored panels, advanced customisation, specialist workflows, bioinformatics and highly complex genomic analysis.

2.5 An integrated approach with distinct roles

Helyx integrates its capabilities through its overall structure. The divisions retain distinct roles: Hyris in distributed PCR, Vytro in clinical PCR and deep multiplexing, and Mytho in custom NGS and bioinformatics. This distinction makes it possible to discuss complementarity without conflating technologies, markets, regulatory constraints or adoption models.

Separating these roles makes roadmaps, terminology, stakeholders and operational responsibilities easier to understand. At the same time, the Group can draw on capabilities shared across its divisions: quality, methods, production expertise, data governance and regulatory awareness.

Mytho provides genomic depth and advanced customisation. Vytro maintains the connection with clinical PCR, IVD where applicable, known targets and laboratory practice, including genetics, oncohaematology and pharmacogenetics, where the choice between PCR and NGS depends on the breadth of the question. Hyris represents distributed access to PCR and the operational management of molecular testing outside centralised settings.

For a molecular biology or genetics laboratory, this structure helps place different needs: routine testing, known targets, selected variants or polymorphisms, clinical PCR panels, distributed testing, custom NGS and bioinformatics. For a partner, it clarifies areas for collaboration. For an institutional stakeholder, it distinguishes regulated settings, distributed models and specialist projects. For those assessing the Group's industrial direction, it explains the relationships among technologies, adoption cycles and execution models.

Chapter 3

Mytho and the value of
NGS:
from genomic complexity
to usable insight

3.1 When NGS creates value

NGS creates value when a complex biological question becomes a manageable result. The technology can read many genomic regions in parallel; its usefulness depends on how well that analysis is designed, controlled, interpreted and communicated.

In many molecular settings, a laboratory must examine dozens or hundreds of genes, identify rare variants, characterise multigene profiles, work with highly heterogeneous cases or build specialist panels for clinical diagnostic programmes, where applicable, research programmes or specialist partnerships. In such cases, NGS provides a broader view than a target-by-target approach and can bring multiple biological hypotheses into a single analytical process, always consistent with intended use and the planned validation model.

This breadth calls for discipline. The final result emerges from a sequence of steps:

- defining the question
- panel design
- sample quality
- library preparation
- sequencing
- quality control
- variant calling
- annotation
- classification
- reporting
- expert review

AMP/CAP recommendations on the validation of NGS bioinformatics pipelines describe the pipeline as an integral part of the assay, to be developed, validated, monitored and versioned according to documented criteria.

The strength of NGS therefore lies in making complexity interpretable and relevant to its intended setting. Mytho contributes Helyx's custom NGS expertise: tailored panels, advanced customisation and bioinformatics for specialist questions.

3.2 Panel design as the first layer of value

An effective NGS process begins with panel design. Before generating data, the question must be translated into a defined genomic scope: which genes or regions to read, at what depth and coverage, with what uniformity and with which stated analytical limits.

This step shapes the entire workflow. A panel poorly aligned with the question can increase noise, uninformative regions, coverage variability and the burden of interpretation. A panel built around the real need strengthens the data and makes downstream bioinformatics more manageable.

Panel breadth must be assessed against the question. A broad panel may be suitable when the biology is heterogeneous or many hypotheses must be examined. In other cases, a more focused panel can produce an output that is easier to interpret, more sustainable and better suited to the workflow. The operational criterion is alignment among the question, targets, coverage, quality and interpretability.

Recommendations for validating NGS panels in oncology state that panel content, the variants to be validated, detection limits and performance metrics must be defined as part of validation. Panel design thus becomes one of the first control points in the overall process.

For Mytho, this step is central. The division works on custom NGS panels, advanced sequencing solutions and optimised bioinformatics pipelines. Its value lies in building tailored projects: panels, advanced customisation and specialist support, with attention to data quality, traceability and alignment with intended use. Any clinical or product-specific performance claims remain tied to the individual application and its validation.

3.3 Data quality and the bioinformatics pipeline

After design, operational value rests on data quality and the pipeline. An NGS workflow produces several layers of information: sequencing reads must undergo quality control, be aligned or processed according to the analysis, converted into interpretable signals, filtered, annotated and reported. Every step involves technical decisions: quality metrics, thresholds, parameters, databases, software versions, inclusion or exclusion criteria and exception handling. Validation of clinical NGS pipelines addresses exactly this issue. Bioinformatics pipelines must be treated as part of the assay and undergo validation, end-to-end testing, monitoring, change control and documentation. Practical recommendations for assembling and validating pipelines for clinical assays likewise call for reproducible, traceable and verifiable processes.

This dimension is less visible than sequencing, but directly affects the robustness of the result. QC, variant calling, annotation, versioning and reporting are technical steps that determine the quality, reproducibility and clarity of the output.

For Mytho, bioinformatics is an essential part of custom NGS value. The division combines genomic and bioinformatics expertise to support custom panels and specialist workflows, making data better organised, easier to read and more relevant to its intended setting. Clinical use of the information remains subject to the available evidence, pathway validation, professional responsibility and expert review.

STEP	Sample and library	QC and sample identity	Variant calling and filtering	Annotation and prioritisation	Classification and reporting	Expert review and updating
KEY QUESTION	Is the starting material suitable?	Are the data robust, traceable and attributable?	Which signals emerge from the data?	What evidence supports those signals?	What can be reported, and within what limits?	Does the result remain consistent with later knowledge and versions?
VALUE	Reduces fragility and upstream variability	Safeguards workflow reliability and control	Turns reads into variants or analytical patterns	Connects data with databases, literature and available knowledge	Produces an output suited to its intended setting	Integrates governance, accountability and reanalysis when needed

Sequencing produces data. An NGS workflow produces a usable result when quality, pipelines, annotation, classification, reporting and expert review are managed consistently.

3.4 Variant interpretation: rules, knowledge bases and expert review

Moving from a variant to its meaning requires rules. In clinical practice, variant interpretation must rest on documented frameworks, evidence and responsibilities. The 2015 ACMG/AMP guidelines introduced a widely used system for classifying germline variants as pathogenic, likely pathogenic, variants of uncertain significance, likely benign or benign, combining genetic, functional, population, family and clinical evidence.

This framework directly affects how NGS becomes usable information. A pipeline can identify a variant; the value of the result depends on placing it within a system of evidence, criteria and limitations. Variants of uncertain significance require caution. Knowledge changes over time: databases, literature and classifications may be updated. Versioning, traceability and the ability to reanalyse therefore become part of data governance.

ClinVar is an important resource in this ecosystem. It is a public archive of variant submissions and interpretations, with information about review status, evidence and updates. Its value lies in structured knowledge sharing; using it calls for attention to the review level, date, context and quality of submissions. A database supports expert assessment but does not replace it.

In this setting, Mytho develops custom NGS workflows in which panels, data and bioinformatics work together to turn complex genomic information into outputs that are clearer, more traceable and more relevant to the question posed by the laboratory, geneticist or partner.

3.5 Mytho: custom NGS and bioinformatics for specialist workflows

Mytho operates in custom NGS. Its role is to support projects that need tailored panels, advanced customisation, compatibility with specialist requirements and dedicated bioinformatics.

The starting point is the need of the specialist laboratory, the geneticist when involved in the pathway, or the industrial or scientific partner: which regions to read, which quality requirements to meet, which platforms to consider, what output to deliver and which limitations to state. This approach reflects the deeper value of NGS: adapting the technology's information capacity to its real setting of use.

Mytho works with next-generation sequencing. Its expertise covers custom NGS panels, advanced customisation, bioinformatics support and specialist workflows, without overlapping with clinical PCR, ready-made IVD products or a standard catalogue model.

Within the Helyx ecosystem, Mytho contributes specialist expertise in broad genomic analysis and the management of data complexity. Vytro provides clinical PCR and deep multiplexing for predefined targets; Hyris makes PCR available in distributed models; Mytho acts where the question requires genomic breadth, custom design and bioinformatics. Clear roles make the specific value of NGS easier to understand.

3.6 Automation, AI and insight governance

Automation and AI can support some parts of the NGS workflow. Within controlled rules and datasets, they can help prioritise findings, retrieve and organise existing evidence, handle large volumes of information and speed up analytical steps. In a complex genomic environment, these tools can reduce operational friction and make available knowledge easier to access.

Their contribution, however, requires governance. Recent reviews of AI and computational methods in variant interpretation identify considerable potential, alongside limitations, bias risks, dependence on training-data quality, difficulties with generalisation and a need for validation before use in sensitive settings. Variants of uncertain significance remain especially delicate: a model can support prioritisation, while responsibility for interpretation remains grounded in rules, evidence and expert review.

For Mytho, the value lies in making the steps leading to and supporting expert review easier to manage: panel design, data quality, the bioinformatics pipeline, traceability, usable output and the updating of knowledge.

Advanced genomics creates value when complexity is organised. An effective custom panel reduces unnecessary breadth. A pipeline developed, validated and monitored according to documented criteria can reduce workflow fragility and variability. Consistent annotation makes the evidence clearer. Expert review safeguards the meaning of the result.

In this space, Mytho strengthens the role of NGS as a specialist capability within the Helyx ecosystem: design, pipelines and data governance serving complex genomic questions.

Chapter 4

PCR and NGS:
diagnostic
appropriateness,
not competition

4.1 The diagnostic question as an operational criterion

The choice between PCR/NAAT and NGS begins with the diagnostic pathway. A laboratory, hospital or public programme needs a useful result, in time to inform a decision, within a sustainable and verifiable workflow.

Every molecular question has a specific technical profile. Looking for a known target calls for a different process from characterising many genomic alterations. Repeated clinical routine testing requires different metrics from specialist investigation. Monitoring a marker over time follows a different logic from building a broad genomic profile.

Appropriateness rests on three dimensions.

The first is the clinical or operational question: confirmation, screening, triage, characterisation, profiling, monitoring or follow-up.

The second is the required output: presence or absence of a target, a defined panel, a multigene profile, an actionable variant, a microbial pattern or a selected biomarker.

The third is the test's place in the workflow: first-line testing, reflex testing, further investigation, surveillance or longitudinal monitoring.

PCR/NAAT and NGS complement each other because they meet different needs. PCR provides a targeted, rapid and repeatable answer when the question is defined. NGS extends the analysis when many signals, genes or alterations, or deeper characterisation, are needed. In real workflows, the two technologies may occupy different stages of the same pathway.

4.2 PCR/NAAT: known targets, first-line testing and routine use

PCR and NAAT have a strong operational profile when the question is narrow. The target is known, the result is needed quickly, the workflow must be robust and repeatability matters more than exploratory breadth.

This logic also appears in institutional algorithms. In tuberculosis diagnostic pathways, WHO guidelines position molecular WHO-recommended rapid diagnostic tests as initial tests in specific scenarios, providing a rapid, standardisable first step. Their value lies in guiding the diagnostic pathway in time for a clinical or public health decision.

In the clinical laboratory, PCR remains central to routine testing, targeted confirmation and monitoring. It is suitable when a laboratory must ask the same type of question across many samples while maintaining quality control, traceability and compatibility with established regulated processes. PCR's maturity creates operational value: protocols, reagents, instruments, controls and skills are already integrated into many diagnostic networks.

Multiplex PCR extends this capability when targets are numerous but predefined. Literature on syndromic panels in clinical microbiology shows how panel-based tests can combine multiple targets in one workflow, potentially improving speed and pathway management, provided use, interpretation and sustainability are carefully governed. A multiplex panel can greatly increase information density while remaining target-driven.

Vytro's deep multiplexing follows this path as the Group's proprietary technology for increasing the information density of PCR across predefined targets. Specific applications remain tied to intended use, applicable validation and the documentation available for each setting.

Vytro provides this clinical laboratory PCR capability: IVD solutions where applicable, deep multiplexing, laboratories, hospitals and defined targets. Hyris comes in when PCR must be distributed across multiple sites or local settings through Hyris System™. In both cases, references to IVD solutions or regulated routine use must be understood within intended use, performance evaluation and applicable requirements. Under the IVDR, clinical evidence connects scientific validity, analytical performance and clinical performance.

4.3 NGS: breadth, heterogeneity and extended characterisation

NGS is appropriate when the question calls for genomic breadth: many genes or alterations, rare variants, actionable biomarkers, biological heterogeneity or cases requiring further characterisation. In such settings, a single-target approach can fragment the pathway.

Advanced oncology offers one of the strongest examples. ESMO recommendations on the use of NGS in patients with advanced cancers place tumour sequencing in specific clinical settings and include the search for tumour-agnostic alterations where matched therapies are accessible. The rationale is practical: genomic profiling becomes part of the pathway when it can support stratification, access to treatment or decisions of major clinical importance.

Clinical genetics provides another example. ACMG guidelines support exome/genome sequencing as a first- or second-tier test in paediatric patients with congenital anomalies, developmental delay or intellectual disability—settings with high aetiological heterogeneity. When many genetic causes could explain the clinical picture, the breadth of testing becomes part of diagnostic value.

NGS requires a multi-step pathway. Sequencing generates the data; a useful result emerges through pipelines, quality control, variant calling, annotation, interpretation and reporting. AMP/CAP recommendations state that bioinformatics pipelines are integral components of NGS assays and must be developed, validated and monitored according to documented criteria. Literature on building and validating clinical pipelines likewise confirms the need for reproducible, versioned and verifiable processes.

Mytho provides this part of the Helyx ecosystem: custom NGS, tailored panels, advanced customisation and bioinformatics. Value lies in panel design, bioinformatics support and the ability to adapt the solution to the needs of the geneticist or laboratory. Bioinformatics and insight make data easier to interpret and manage; clinical decisions remain tied to context, available evidence and the responsibilities assigned within the pathway.

Diagnostic question	Most appropriate technology	Operational rationale	Helyx
Do I need to confirm one or a few known targets quickly?	PCR	Targeted, repeatable result suited to routine use and short turnaround times.	Vytro / Hyris, depending on setting
Do I need to test many predefined targets in one process?	Multiplex / deep multiplexing PCR	Greater information density within a target-driven approach.	Vytro
Do I need to work in distributed or multisite settings?	Distributed PCR	Operational deployment, remote management, proximity to the decision point.	Hyris
Do I need to examine many genes or alterations?	NGS	Genomic breadth, parallel analysis and richer information.	Mytho
Am I facing a case with high genetic heterogeneity?	ES/GS or broad NGS panels	Broad scope and many simultaneous hypotheses.	Mytho
Do I need to characterise a confirmed case or an already positive sample?	NGS for further investigation / follow-on testing	Sequencing for resistance, variants, profiling or genomic characterisation.	Mytho, with upstream PCR/NAAT
Do I need to profile a complex microbial community?	NGS / metagenomics	Broad analysis of composition and biological patterns.	Mytho
Do I need to follow selected targets over time?	PCR or targeted quantitative methods	Focused measurement, follow-up and possible applied translation.	Vytro / Hyris for validated PCR

4.4 Integrated workflows: detect, investigate, characterise

Many modern diagnostic pathways follow a sequence of decisions. A workflow may begin with rapid detection, proceed to further investigation and end with monitoring or follow-up. PCR/NAAT and NGS can play different roles in this sequence.

Tuberculosis is a particularly useful example. In WHO pathways, a rapid molecular test may sit at the start of the algorithm, while targeted NGS may be used subsequently for additional characterisation, especially when several genes or regions relevant to resistance profiles must be read. The first tier directs the pathway; the next increases its information resolution.

A similar pattern has emerged in genomic surveillance of respiratory viruses. In various SARS-CoV-2 monitoring programmes, RT-PCR-positive samples were selected for sequencing to characterise variants and support epidemiological surveillance. PCR supports detection and operational throughput; sequencing adds genomic depth and classification capability.

These examples show how PCR and NGS can belong to the same pathway without duplicating each other. PCR can provide a rapid, standardisable entry point. NGS can add breadth, characterisation or extended genomic information when deeper investigation is needed. In other settings, the pathway begins directly with NGS because the clinical question is broad from the outset. The criterion remains the intended context of use.

4.5 Microbiota: broad profiling and targeted measurement

A complex microbial community often calls for a broad analysis of composition, diversity, biological patterns, relationships among taxa and functional signals. At this stage, NGS and metagenomics provide information suited to profiling and exploration.

Methodological literature on microbiome analysis shows that study design, sample collection, processing, choice of analytical methods and interpretation all affect profile robustness. After broad profiling, PCR can support more focused measurements of selected targets, provided the test design is validated and reported using appropriate quality criteria.

Microbiota analysis should be understood as an example of methodological integration, not a general clinical promise. The sequence is clear: broad profiling, signal selection, targeted measurement and possible applied translation. Mytho contributes NGS analysis and bioinformatics; Vyto enters regulated clinical pathways when the question concerns defined targets and PCR assays consistent with their intended use; Hyris may be relevant to distributed or non-clinical OEM models in which PCR needs to be brought closer to the operational setting.

4.6 Implications for Helyx Industries S.p.A.

Diagnostic appropriateness is also an industrial proposition. Helyx Industries S.p.A. organises different technologies into distinct areas, each linked to specific questions, markets and workflows.

Vyto is the division most directly aligned with clinical PCR: laboratories, hospitals, IVD solutions where applicable, deep multiplexing, panels and regulated routine testing. It is the most appropriate response when the question concerns known targets, standardisation, repeatability and operational value in the laboratory.

Mytho provides the custom NGS capability: tailored panels, advanced customisation, bioinformatics and support for highly complex settings. It addresses questions requiring genomic breadth, many targets, biological heterogeneity or extended characterisation.

Hyris retains its role in distributed PCR architecture and Hyris System™: multisite deployment, local settings, remote management and processes in which proximity to the decision point reduces operational friction.

The Group's strategic value comes from the clarity of these roles. Each technology is placed where it can create diagnostic and industrial value. For customers, hospitals and laboratories, this structure helps identify the most suitable pathway. For partners and institutional stakeholders, it clarifies opportunities for collaboration. For industrial and financial stakeholders, it makes markets, adoption cycles and execution models easier to understand.

Advanced molecular diagnostics grows when technology follows the question. Known targets, routine testing, follow-up, genomic breadth, characterisation and interpretation belong to different stages of the workflow.

Helyx Industries S.p.A. addresses this evolution through a structure of complementary capabilities: distributed PCR, clinical PCR with deep multiplexing and custom NGS. The model's strength lies in keeping roles distinct while also building integrated pathways when the diagnostic question requires them.

Broad profiling

NGS / metagenomics



Signals of interest

taxa, patterns, markers



Targeted measurement

PCR



Follow-up

selected targets, defined setting

In microbiota analysis, NGS and PCR can contribute at different stages of the same pathway: profiling complexity and targeted measurement of selected signals.

Chapter 5

NGS and genomic newborn screening: the public Genoma-Puglia initiative

5.1 Genomic newborn screening: a high-responsibility application

Newborn screening is one of the fields in which molecular diagnostics carries the greatest responsibility for health. A result does more than identify a variant or biological signal: it may guide confirmation, counselling, care and follow-up. When sequencing enters this setting, the range of information expands, as do requirements for consent, data management, diagnostic confirmation and integration into clinical pathways.

Genomic newborn screening is being explored in several international programmes as a possible extension of traditional screening, especially for rare or serious conditions that may be affected by early intervention. Its introduction requires rigorous criteria: transparent selection of conditions, gene-disease evidence, consent, data quality, diagnostic confirmation, follow-up and governance.

This framework helps explain the role of NGS. The value of sequencing depends on turning a public health question into a manageable process: selection criteria, sample quality, bioinformatics workflow, traceability, interpretation, diagnostic confirmation and care. Sequencing is one part of the pathway; a screening programme requires a broader health, technical and organisational framework.

Genoma-Puglia allows us to observe this transition in a documented institutional setting. For Helyx Industries S.p.A., the case shows how custom NGS expertise can contribute to highly complex programmes in which the panel, data quality and bioinformatics must meet defined requirements.

5.2 Genoma-Puglia: a documented institutional programme

Genoma-Puglia began as a regional programme with a legal basis, located at the Genomic Medicine Laboratory of the Di Venere Hospital. It concerns genomic profiling of newborns and the possible early identification of genetically determined conditions, with voluntary participation and informed parental consent.

The initiative therefore operates within an institutional framework. It arose from a regional decision, involves a public reference laboratory and takes place in the highly complex field of newborn screening. Governance, consent and the healthcare pathway become integral to the project's value.

Over time, Genoma-Puglia has progressed from an initial research and experimental phase towards broader application in the regional health system. The programme analyses newborn DNA to identify more than 400 relevant and treatable metabolic disorders and rare diseases early, so that clinical and/or therapeutic intervention may be possible.

Its importance lies in the model it reveals: genomic newborn screening requires a connection between condition-selection criteria, sample quality, bioinformatics, diagnostic confirmation and integration into clinical pathways. Genoma-Puglia is thus a useful example of the organisational complexity of NGS within an institutional programme.

5.3 Revvity, Helyx and the structure of their collaboration

Revvity Italia was awarded the public tender to supply tests and instruments for the Genoma-Puglia project. Under its agreement with Revvity Italia, Helyx Industries S.p.A. develops, produces and supplies customised NGS-based screening kits made to the partner's requirements.

The collaboration clearly distinguishes the roles:

- the programme remains the responsibility of the healthcare and institutional bodies involved;
- Revvity Italia operates within the supply arrangement established by the tender;
- Helyx contributes the development and production of custom NGS kits.

Mytho provides the Group expertise most directly related to the project: tailored panels, advanced customisation, bioinformatics support and specialist workflows.

The case shows how Helyx's NGS capability can be applied to a public programme in which data quality, documentation and compliance with technical requirements are essential.

Screening

Genomic newborn screening is a programme, not a single test. The value of NGS depends on connecting selection criteria, quality, interpretation, confirmation and governance.

5.4 From technology to programme: why NGS requires governance

In genomic newborn screening, complexity arises both before and after sequencing. The technology enables broad analysis, but a programme's value depends on how conditions are selected, data managed, results communicated and subsequent pathways organised.

The first decision concerns which conditions to include. The most structured international initiatives use explicit criteria: gene–disease evidence, severity, the possibility of early intervention, whether a result can be confirmed and access to care. Condition selection thus becomes the first level of programme governance.

A second level concerns consent and communication. Genomic sequencing can generate complex information, including uncertain results or findings that do not immediately lead to clinical action. Consent, communication with parents and expectation management are therefore central to the pathway, not optional extras.

The technical and organisational dimension is equally important. Samples, data quality, the bio-informatics pipeline, reporting and traceability must support a repeatable process. NGS creates value when data can become an output that is manageable, documentable and aligned with the programme.

Finally, a positive screening result requires a subsequent pathway: confirmatory testing, specialist assessment, possible genetic counselling and clinical follow-up. This distinction keeps screening separate from diagnosis and prevents sequencing from being treated as a self-sufficient answer.

Dimension	Question to govern	Risk avoided / value for NGS
Condition selection	Which conditions are relevant, treatable or improvable if identified early?	Avoids broad panels poorly aligned with clinical and programme criteria.
Consent and communication	What is being sought, what is excluded, and what uncertainties may arise?	Places testing within a proportionate information pathway.
Data quality	Are the sample and data robust, traceable and repeatable?	Makes QC, coverage, pipelines and reporting part of the value.
Interpretation	How can variants and signals become manageable outputs?	Requires annotation, filtering and expert review.
Diagnostic confirmation	What happens after a positive screening result?	Keeps screening, confirmation and care distinct.
Public governance	How are data, equity, follow-up and sustainability managed?	Connects technology, accountability and the health system.

From programme to governed result

Selection criteria



Custom NGS panel



Data quality



Bioinformatics pipeline



Documentable output



Confirmation and care

In genomic newborn screening programmes, the value of NGS depends on connecting selection criteria, data quality, bioinformatics, documentable output and confirmation pathways.

5.5 An industrial reading of the case: Mytho and custom NGS

Genoma-Puglia fits high-responsibility B2B settings in which the panel, data quality and workflow must be documented. The strength of the case does not lie in the number of targets examined, but in the quality of the process that makes a panel usable within a structured programme.

A custom NGS model matters when a project needs a panel aligned with programme criteria, a repeatable process, technical documentation, traceability, interpretable output and integration with later clinical steps. In this setting, value comes from designing for specific requirements and adapting technology, data quality and bioinformatics support to the project's question.

Mytho provides this capability within Helyx: custom NGS, tailored panels, advanced customisation, bioinformatics support and specialist workflows. Genoma-Puglia places this capability within Helyx's industrial direction, showing how the company participates in complex NGS projects through an already documented supply collaboration.

The company's role remains clear: to contribute the custom NGS component of a highly complex project while keeping technology supply separate from the clinical and institutional management of the programme.

5.6 Strategic implication: NGS as an applied specialist capability

Genoma-Puglia reveals a stage in NGS maturation. The technology is part of a programme in which every step must be governed: condition selection, panel design, sample quality, pipelines, reporting, confirmation and healthcare integration.

For Helyx Industries S.p.A., the case's strategic value lies in its alignment with Mytho's expertise. Custom panels, advanced customisation and bioinformatics matter when NGS enters structured programmes with defined requirements and responsibilities shared among institutional, clinical and industrial parties.

The case highlights requirements that are increasingly important in complex NGS projects: reliable data, traceability, manageable output, documentation and integration into healthcare pathways. Genomic newborn screening is especially demanding because it brings together technology, public health, ethics, clinical practice, industry and governance.

Applying this model elsewhere requires selected settings, clear criteria and governed pathways. In programmes of this kind, NGS can become an applied specialist capability, provided the panel, data quality, confirmation and healthcare integration remain clearly defined.

For Helyx, Mytho is the capability most directly connected to this direction: designing documentable, usable custom NGS pathways in which value extends beyond data generation to building a workflow aligned with the programme's question.

Chapter 6

Microbiota:
NGS + PCR synergy
in understanding
biological complexity

6.1 The microbiota as a dynamic community

The microbiota is a complex, dynamic biological community that is sensitive to context. Anatomical site, sample type, collection method, processing, analytical method and bioinformatics pipeline all contribute to the profile observed. Every result therefore arises from an interaction between biology and method.

This makes the microbiota an effective example for understanding how NGS and PCR complement each other. When the question concerns the overall structure of a microbial community, sequencing can explore composition, diversity, patterns and relationships among taxa. When attention turns to specific signals, selected targets or targeted measurements, PCR can add a more focused layer.

Best practices for microbiome analysis emphasise the importance of study design, sampling, extraction, sequencing technology, databases, bioinformatics analysis and interpretation. Multi-centre studies and comparisons of metagenomic strategies confirm that workflow choices can substantially affect the observed profile and the comparability of results.

The question guides the workflow. Sometimes a broad picture of the microbial community is needed. In other cases, a specific target must be measured. Elsewhere, an identified signal must be tracked over time. The most suitable technology depends on the sample, setting and intended use of the result.

6.2 Profiling complexity: the role of NGS

NGS technologies allow the microbiota to be examined more broadly than approaches focused on individual targets. Metabarcoding, such as marker-region analysis, can describe the taxonomic composition of a microbial community. Shotgun metagenomics can extend the analysis to the sample's genetic content and indicate the community's functional potential, depending on sample quality, sequencing depth, the pipeline and available databases.

This breadth matters when the question is exploratory or the biological system is too variable to reduce to a few initial targets. In a microbiota profile, a laboratory may want to know which taxa are present, how diversity changes, which patterns appear across sample groups or which signals merit later, more focused measurement.

Mytho contributes Helyx's custom NGS and bioinformatics expertise in this field. Profiling microbial communities involves more than generating sequences: method selection, quality control, database selection, pipelines, filters and delivery of an output aligned with the question.

NGS offers a broad view. In this setting, its strength is making a complex community observable and revealing signals that may not be known in advance. A profile becomes truly useful when it is developed within a sound analytical design and linked to a defined question.

Two levels of microbiota analysis
The information available depends on the method and the quality of the entire workflow, from the sample to the bioinformatics pipeline.

Metabarcoding / 16S	Shotgun metagenomics	Methodological caution
Describes the taxonomic composition of the microbial community.	Extends analysis to the sample's genetic content and functional potential.	The sample, extraction, sequencing depth, databases and pipeline affect the profile observed.

6.3 From signals to targets: where PCR comes in

Broad profiling may reveal a more focused question. A taxon, group of microorganisms, marker or pattern can become the subject of targeted measurement. At this stage, PCR can complement NGS by examining selected targets with greater operational precision.

Sequencing helps describe the community; PCR can track defined signals when more focused, standardised measurement is needed within a controlled assay. It can also support targeted quantification of specific targets when the assay is calibrated, controlled and validated for the matrix, target and intended use.

Targeted measurement has value when it is built around an assay suited to the target, matrix and intended use of the result. It does not automatically turn an NGS profile into a clinical conclusion; it makes a selected signal measurable in a more focused and controlled way.

In a molecular biology or genetics laboratory, the same user may move between different questions. Broad analysis may be needed when the system is complex, and targeted measurement when the target is defined. This continuity makes the complementarity of NGS and PCR concrete: different tools for different levels of the same question.

Stage	Question	Primary technology	Caution
Broad profiling	How is the microbial community organised?	NGS / metabarcoding / metagenomics	The profile depends on the sample, method, databases and pipeline
Profile analysis	Which taxa, patterns or signals merit attention?	Bioinformatics and profile analysis	The signal needs context and should not automatically become a clinical conclusion
Selected target	Which signal needs targeted measurement?	PCR	The target must be defined and the assay controlled
Quantitative measurement	Should relative analysis be supplemented by more focused measurement?	PCR or a validated quantitative workflow	Calibration, controls, the matrix and clear intended use are needed
Use of the result	Are the data appropriate for the intended setting?	Validation, application context and governance	This pathway is not a universal standard

Note: this pathway illustrates one possible analytical approach, not a universal standard. Each step depends on the sample, target, assay, pipeline and intended use.

6.4 The quantitative challenge

One of the main complexities in microbiota analysis concerns the nature of the data. Many sequencing outputs describe relative abundance: the proportion of one taxon compared with others in the analysed sample, without necessarily indicating the absolute quantity in the starting material. Literature on compositional data treats this as a fundamental aspect of microbiome analysis.

Relative abundances can be informative but require caution. One taxon may appear proportionally more abundant because another has decreased. Two samples may have similar compositions but different microbial loads. Extraction, primers, regions analysed, sequencing depth, contamination and databases can all affect the observed output.

For some questions, adding a quantitative layer to compositional profiling can provide useful information. Quantitative microbiome profiling has shown that connecting community composition with microbial load can make variation within and between individuals easier to understand. This principle does not prescribe one technical solution; rather, it shows that relative analysis can benefit from quantitative information generated by appropriate, controlled methods.

In this setting, PCR can complement NGS for selected targets. Targeted measurement can help track or quantify a defined signal, without by itself resolving the limitations of the sample, matrix or workflow.

Complementarity arises from the difference between the two information levels: NGS opens a view onto complexity, while PCR can focus on selected targets.

6.5 Validation, intended use and limits of microbiota analysis

The microbiota is a field with considerable potential, but translating findings into research applications, industrial control, professional workflows or possible regulated devices calls for caution. A microbiota profile does not have the same meaning in an exploratory project, quality control, a professional laboratory or a regulated pathway.

Intended use defines the value of a result. A research application may address more exploratory questions. An industrial or distributed application may prioritise robustness, repeatability and operational simplicity. In a possible regulated setting, a test would require evidence, validation, analytical standards and governance appropriate to its intended use, distinguishing scientific validity, analytical performance and clinical performance.

Recent literature on commercial microbiota tests in Europe identifies a real risk: premature interpretation, insufficient standardisation, variable methodological transparency, excessive claims and difficulty distinguishing exploratory analyses from products with a regulated purpose.

The value of microbiota analysis depends on the question, setting, method quality and available validation. Profiling, targeted measurement, monitoring and applied follow-up are useful when linked to a coherent workflow with stated limitations and a clear intended use.

6.6 Implication for Helyx: clear complementarity

The microbiota shows why advanced molecular analysis often requires an ecosystem of technologies rather than a single platform. A complex microbial community may first require a broad analysis. That analysis can reveal signals to measure more precisely. Workflow value depends on linking the profile, targets and intended setting without turning an analytical signal into an unsupported conclusion.

MYTHO 

Mytho

Contributes Helyx's custom NGS and bioinformatics expertise. Its role is to support broad, controlled analysis of complexity, with pipelines and outputs built around the analytical question.

VYTRO 

Vytro

Comes in when the question concerns laboratory PCR, defined targets or focused molecular panels, always according to intended use and applicable validation. In molecular biology and genetics laboratories, PCR and NGS may serve the same user: NGS when breadth and depth are needed, PCR when the question concerns a few targets or selected markers.

HYRIS 

Hyris

Adds another layer when PCR must be organised in distributed, multisite or decentralised settings. Here too, value depends on selected targets, controlled assays and a defined context of use.

The microbiota case does not describe a universal solution, but it illustrates an integrated approach: use NGS when breadth is needed, use PCR when focus is needed, and let validation, context and intended use determine the result's real value.

For Helyx, this reading is consistent with an ecosystem in which different technologies address different questions, without overlap or undue generalisation.

Chapter 7

The strategic value of NGS:
building expertise, work-
flows and credibility now

7.1 A more accessible technology that still demands expertise

NGS is entering a phase of industrial and applied maturation. Generating sequencing data has become progressively more accessible, supported by lower costs, evolving platforms and a growing pool of expertise. This change has expanded the settings in which sequencing can be considered: translational research, precision oncology, clinical genetics, public genomics programmes, selective screening and specialist workflows.

Greater accessibility, however, concerns only part of the process. In real laboratory practice, NGS includes the sample, library preparation, panel choice, sequencing, quality control, the bioinformatics pipeline, annotation, interpretation, reporting, data governance and updates to knowledge. The cost of data generation has fallen substantially; the complexity of the complete pathway remains significant.

This is where NGS maturation takes place. As data generation becomes more accessible, value shifts towards what makes those data reliable in practice: analytical quality, robust pipelines, current databases, versioned processes, expert review and alignment with the intended setting. NGS is mature enough for use in specific clinical, institutional and specialist settings, while still demanding substantial expertise. This is the stage at which methods, technical reputation and operational capability are built.

7.2 Adoption grows in highly complex settings

NGS enters diagnostic processes primarily where the biological or clinical question goes beyond a few known targets. Its value emerges when many genes, regions or alterations, or highly heterogeneous profiles, must be examined.

In advanced oncology, specialist recommendations identify settings in which genomic profiling can support stratification, identify actionable alterations or help access targeted therapies, always within specific clinical indications. In clinical genetics, exome and genome sequencing have been recommended for highly heterogeneous paediatric presentations such as congenital anomalies, developmental delay or intellectual disability, where many genetic hypotheses may be relevant.

Public genomics programmes point in a similar direction. The most structured initiatives do more than introduce technology: they build networks, test directories, eligibility criteria, data governance, interpretation processes and shared organisational models. Genomics becomes part of health systems when supported by infrastructure, rules and defined responsibilities.

Oncology, clinical genetics, selected genomics programmes and microbiota research, despite very different levels of maturity, illustrate the same principle: sequencing matters when the required result is not a single answer but a broader, governed analysis of biological complexity. Each setting must be assessed in light of its indication, intended use, workflow and available evidence.

7.3 From data to workflow: where value is created

As NGS matures, attention shifts from data to workflow. Producing a sequence is one stage; making the data reliable, interpretable and usable requires a series of technical decisions.

Panel design affects coverage, depth, the regions examined and clarity of the result. Sample and library quality determine what can be observed. The bioinformatics pipeline converts reads and signals into variants, patterns or structured outputs. Annotation, filtering, classification and reporting connect data with available knowledge. Versioning, benchmarking and change control support reproducibility and traceability.

AMP/CAP recommendations on validating NGS bioinformatics pipelines describe the pipeline as an integral part of the assay. Literature on practical validation of clinical pipelines also calls for documented processes, end-to-end testing, monitoring and change management. In this setting, clinical bioinformatics becomes an organisational capability combining software, quality, biological knowledge, governance and responsibility for the result.

When a pathway enters diagnostic or regulated settings, intended use, analytical validation, clinical performance where applicable and data governance become part of documented responsibility. More accessible sequencing technology creates opportunities; a robust workflow makes those opportunities usable.

Why timing matters

NGS maturation is visible in several signals: more accessible data, selective adoption, public networks, bioinformatics and progressive standardisation. Each signal requires careful interpretation.

Sign of maturation	What it indicates	How to interpret it
Lower data-generation costs	Sequencing becomes more accessible	The complete pathway includes bioinformatics, interpretation and governance
Adoption in selected clinical settings	NGS enters where complexity calls for breadth	It does not amount to universal routine use
Public genomics programmes	Technology requires networks, criteria and shared processes	Implementation depends on governance and sustainability
Central role of bioinformatics	Value shifts towards pipelines, annotation and reporting	Data do not automatically produce insight
Growing standardisation	Processes become more repeatable	Methods and credibility must be built before full maturity

7.4 Building credibility before full standardisation

Credibility takes time in highly complex diagnostic markets. It is built through expertise, applications, partner relationships, operational quality, dialogue with laboratories and the ability to answer specific questions.

NGS is particularly sensitive to this dynamic. Every project needs alignment among the question, panel, data quality, pipeline, output and intended use. Experience from real cases helps clarify which questions need a custom panel, which pathways are sustainable, which steps can introduce weaknesses and which outputs laboratories, geneticists or partners can actually use. This work takes place while use of the technology is widening but still requires considerable expertise. This is when methods, technical reputation, the quality of partner relationships and the ability to manage complex analytical pathways are established.

Strategic value does not come from proclaiming technological superiority. It comes from building a credible presence in a field that demands adaptation, method and trust.

7.5 Mytho as NGS matures

Mytho participates in this phase as the custom NGS division of Helyx Industries S.p.A. Its role fits projects in which the panel, bioinformatics and workflow must be adapted to the question posed by the laboratory, geneticist or partner.

This positioning makes sense because many NGS applications are not yet fully standardised. Some questions call for a tailored panel, a targeted choice of regions or genes, a pipeline suited to the data type and an output compatible with the project. Advanced customisation becomes an industrial capability when connected to design, control, documentation and data quality.

Mytho works in this space: panel design, data quality, bioinformatics and support for specialist workflows. Its value is not a complete clinical platform or a standard catalogue applied indiscriminately. It lies in addressing complex NGS questions and making genomic depth understandable, documentable and usable.

Applications illustrate two directions. On one hand are institutional programmes in which NGS requires criteria, quality and governance. On the other are integrated workflows in which broad profiling can be followed by more targeted measurements. In both cases, genomic data gain value within a carefully designed pathway.

7.6 The Helyx ecosystem avoids a false choice between NGS and PCR

Strengthening NGS does not diminish the role of PCR; it makes that role clearer.

In laboratory practice, the choice between PCR and NGS depends on the breadth of the question and the downstream decision. When a few targets or known variants are involved, PCR remains a focused answer compatible with established workflows. As the number of genes, regions or alterations to examine grows, NGS becomes more appropriate. The laboratory, geneticist and clinical or scientific partner may remain the same stakeholders; the depth of the question and the required output change.

This continuity makes the Helyx structure easy to understand. Mytho provides genomic depth, custom panels and bioinformatics. Vytro retains the connection with clinical PCR, laboratories, defined targets, oncohaematology, pharmacogenetics and molecular genetics. Hyris maintains distributed PCR and Hyris System™, useful when testing must be organised closer to the decision point or across multiple sites.

The ecosystem's value lies in clear roles. Laboratories, partners and institutional stakeholders can distinguish when genomic depth, focus on known targets or distributed execution is needed. The evolution of molecular diagnostics does not call for a single technology; it calls for selecting the tool that best suits the question.

7.7 An industrial path to build now

NGS is at a stage where technological maturation and operational complexity coexist. Data generation is more accessible; adoption is growing in selected settings; public programmes show governance models; bioinformatics is increasingly central. Yet the full pathway remains highly dependent on expertise.

This is the time to build capability: through well-designed panels, documentable workflows, applications, data quality, bioinformatics, dialogue with laboratories and partners, and integration with PCR when the question calls for it, rather than through market declarations.

Helyx Industries S.p.A. addresses this path through a coherent structure of capabilities. Mytho supports genomic depth and custom NGS projects. Vytro maintains the connection with clinical PCR and defined targets. Hyris adds distributed PCR. Together, these areas make advanced molecular diagnostics a system of appropriate choices.

The strategic moment for NGS should not be read as a market promise. It is a period of building. Data generation is becoming more accessible, but operational translation remains complex. For Helyx, value lies in managing this complexity through clear roles: genomic depth when breadth is needed, clinical PCR for defined targets and distributed PCR when the setting demands operational proximity. The aim is a system of choices aligned with the question.

Conclusion

Molecular diagnostics is moving towards a greater ability to select, combine and govern different technologies according to the question.

PCR has already proved its maturity as a focused, repeatable technology integrated into laboratory workflows. NGS is maturing as a tool for broad analysis, useful when genomic complexity calls for extensive panels, bioinformatics, interpretation and data governance. The distinction between the technologies defines a criterion for appropriateness.

When a question involves a few known targets, variants or polymorphisms, PCR provides a sound operational pathway. When it involves many genes or regions, high heterogeneity or deeper characterisation, NGS is more suitable. In various settings the two technologies can be part of the same workflow: one to analyse complexity, the other to measure selected targets or support targeted follow-up.

Helyx Industries S.p.A. organises this approach into an ecosystem with distinct roles. Mytho brings the Group into custom NGS, tailored panels and bioinformatics. Vytro provides clinical PCR and deep multiplexing in molecular biology and genetics laboratories, where known targets, routine testing and targeted investigations remain essential. Hyris retains distributed PCR, useful when operational proximity and multisite deployment become part of the value.

The applications make this structure concrete. Genomic newborn screening shows the complexity of NGS in institutional programmes, where technology, clinical criteria, quality and governance must come together. Microbiota analysis shows how broad profiling can generate signals to examine, select and measure with more focused tools. In both cases, value comes from how technology is placed within a usable pathway.

Strengthening Helyx's NGS capability has value at this stage of maturation. Sequencing data have become more accessible to generate, but the complete pathway still demands substantial expertise. Building capabilities, workflows, applications and credibility now means preparing for a setting in which genomics will become progressively more integrated into laboratories, public programmes and specialist projects.

The direction is clear: use PCR and NGS precisely, according to the question and setting. Advanced molecular diagnostics requires breadth when complexity demands it, focus when the target is defined and distribution when the operational setting calls for it.

Helyx approaches this evolution through an industrial structure that connects genomic depth, molecular routine testing and distributed accessibility as complementary parts of one vision.

Why does a White Paper on NGS also discuss PCR?

Because NGS does not operate in isolation. In real laboratories and applied programmes, different technologies address different questions. PCR remains central when the target is known, the answer must be focused and the workflow requires repeatability. NGS becomes relevant when genomic breadth, many genes, rare variants, custom panels or a deeper understanding of biological complexity is needed.

Will NGS replace PCR?

No. Appropriateness is the right principle. PCR is effective when the question is narrow; NGS is better suited to questions requiring breadth. In many pathways the two technologies can work together: broad analysis may be followed by targeted measurements, or a rapid test by deeper genomic investigation.

When is PCR more appropriate, and when is NGS?

PCR is more appropriate when a laboratory must examine a few known targets, variants or polymorphisms in a focused, repeatable workflow. NGS is more appropriate when the number of genes, regions or alterations to examine grows substantially, or when the question concerns complex profiles, biological heterogeneity or extended characterisation.

What is Mytho's role in the Helyx ecosystem?

Mytho represents Helyx's custom NGS capability. It works on tailored panels, advanced customisation, bioinformatics and specialist workflows. Its value is especially relevant when a question cannot be met by a standard catalogue but calls for panel design, data quality and bioinformatics support suited to the intended setting.

How does Vytro's role differ from Mytho's?

Vytro provides clinical PCR and deep multiplexing in molecular biology and genetics laboratories. In fields such as genetics, oncohaematology and pharmacogenetics, PCR and NGS may serve the same stakeholders: PCR when a few targets or defined variants are needed, NGS when the question requires broader analysis.

Where does Hyris fit?

Hyris brings PCR into distributed, multisite and operationally flexible models through Hyris System™. Its role matters when molecular testing must move closer to the point of use while maintaining workflow control, traceability and result management.

Why is Mytho not presented as a standard catalogue?

Because in complex settings the value of NGS often comes from adapting the panel, workflow and bioinformatics to the specific question. Mytho fits a custom model: tailored panels, specialist support, data quality and bioinformatics built around the project.

What makes Genoma-Puglia strategically significant?

Genoma-Puglia shows NGS in a highly complex institutional setting, where technology, selection criteria, quality, governance, diagnostic confirmation and integration into clinical pathways must come together. The case's value is not the number of targets examined, but the ability to place sequencing within a structured, documentable programme.

Why is the microbiota a good example of NGS + PCR synergy?

The microbiota is a complex biological community. NGS can provide broad profiling of the

microbial community; PCR can come in when selected targets need more focused measurement, tracking or monitoring. Value always depends on the sample, method, validation, setting and intended use.

Why is timing relevant for Helyx?

Generating NGS data is more accessible, but the complete pathway remains complex. Building expertise, applications, workflows, bioinformatics and relationships with laboratories and partners now prepares Helyx for a stage in which genomics is increasingly integrated into specialist projects and health programmes.

Why is bioinformatics so central to NGS?

Because the value of NGS is more than generating sequences. Sequencing data must be checked, processed, filtered, annotated and turned into an understandable output. Bioinformatics pipelines, data quality, versioning, databases and reporting are structural parts of the NGS pathway. Without them, the technology's information depth is difficult to use reliably.

What does "intended use" mean in this White Paper?

Intended use is the setting for which a test, panel or workflow is designed, validated and used. The same technology can have different meanings in research, a clinical laboratory, an institutional programme or an industrial setting. The paper therefore always links a result's value to its setting, available validation and the responsibilities defined by the pathway.

How should Vytro's deep multiplexing be understood?

Vytro's deep multiplexing belongs to the clinical PCR, target-driven part of the Helyx ecosystem. Its value lies in increasing the information density of PCR across predefined targets while retaining a targeted answer. It therefore differs from NGS, which is used when the question calls for genomic breadth, many genes or regions, or more extensive characterisation.

What is the main limitation when discussing the microbiota?

The microbiota should not be presented as an automatic diagnosis or as a direct basis for general clinical, nutritional or therapeutic recommendations. Profiling can provide relevant information, but its value depends on the sample, method, pipeline, validation, selected targets and intended use. The paper uses it as an example of an integrated workflow, not a universal clinical promise.

What does building credibility in NGS mean?

It means accumulating expertise, applications, operational quality, documentable workflows and dialogue with laboratories, geneticists, partners and institutional stakeholders. NGS data are becoming more accessible to generate, but the complete pathway remains complex. Credibility at this stage is built by demonstrating the ability to design, control and make genomic complexity usable.

Appendix B

Essential glossary

Actionable alteration

A molecular alteration that may be relevant to a clinical decision, stratification, further investigation or a treatment pathway, depending on evidence and intended use.

Analytical performance

A test's technical ability to detect or measure the intended target correctly against defined analytical parameters.

Annotation

The process of linking variants or molecular signals to information in databases, literature and knowledge bases.

Diagnostic appropriateness

The principle of selecting a technology according to the question, required output, intended setting and workflow.

Assay

An analytical procedure designed to detect, measure or characterise a specific target or set of targets under defined technical conditions.

Bioinformatics

Computational processes that turn molecular or genomic data into clearer analytical outputs: quality control, alignment, variant calling, annotation, filtering, reporting and data management.

Clinical performance

A test's ability to provide clinically relevant information in relation to its stated intended use.

Coverage

The extent to which sequencing reads a genomic region. Adequate coverage is essential for reliable analysis of regions of interest.

Advanced customisation

The ability to adapt a panel, workflow and bioinformatics support to specialist needs rather than relying on an undifferentiated standard catalogue.

Compositional data

Data expressed as relative proportions among components. In microbiome profiles, relative abundance does not necessarily equal the absolute quantity in a sample.

DBS – Dried Blood Spot

A blood sample dried on absorbent paper, frequently used in newborn screening programmes.

Deep multiplexing

A proprietary PCR approach designed to increase information density across predefined targets. In the Helyx ecosystem, it is associated with Vytro.

Depth / read depth

The number of times a genomic position is read during sequencing. It affects the ability to detect variants and signals reliably.

Intended use

The context and purpose for which a test, workflow or device is designed, validated and used. It defines requirements, expected performance and responsibilities.

Follow-up

Subsequent monitoring of a patient, target or signal over time, according to the clinical, laboratory or applied setting.

Genomic newborn screening

The use of sequencing in newborn screening programmes to identify selected conditions early, with criteria for governance, diagnostic confirmation, consent and care.

Data governance

Rules, processes and responsibilities that ensure traceability, quality, versioning, security, updating and appropriate use of data.

IVD – In Vitro Diagnostic

A diagnostic device or test intended for use on human biological samples to provide clinical information, subject to regulatory requirements and its stated intended use.

Library preparation

The sample-preparation stage in which genetic material is converted into a library compatible with the sequencing platform.

Metabarcoding / 16S

A sequencing approach based on marker regions, often used to describe the taxonomic composition of microbial communities.

Metagenomics

Analysis of the overall genetic material in a microbial community to profile its composition and functional potential.

Microbiota

The community of microorganisms present at a particular biological site or in an environment. Its study requires attention to the sample, method, pipeline and interpretive context.

NAAT – Nucleic Acid Amplification Test

A category of tests based on nucleic acid amplification, including PCR and related technologies.

NGS – Next-Generation Sequencing

Parallel sequencing technologies that can read many genomic regions, panels, exomes, genomes or microbial communities within one analytical workflow.

Custom panel

A panel designed to specific requirements by selecting genes, regions or targets relevant to a clinical, research or project question.

PCR – Polymerase Chain Reaction

A nucleic acid amplification technology used to detect defined genetic or molecular targets. It is particularly suitable for targeted questions, routine testing and follow-up of known targets.

Performance evaluation

Assessment of evidence for the scientific validity, analytical performance and clinical performance of a diagnostic test or device.

Bioinformatics pipeline

A documented sequence of software and analytical steps for processing NGS data, from raw data to an interpretable output.

Genomic profiling

Analysis of a broad set of genes, regions or alterations to provide a more extensive molecular picture than a single target.

Quality Control / QC

Controls used to check sample quality, workflow performance, data robustness and output reliability.

Reflex test

A subsequent test performed in response to an initial result to investigate, confirm or better characterise the first signal.

Reporting

The stage in which an analytical result is structured into an output that is understandable and appropriate to its intended setting.

Sample-to-result

The complete pathway from biological sample to final result, including preparation, analysis, quality control, bioinformatics, interpretation and reporting.

Scientific validity

A scientifically established relationship between a marker, variant, target or biological condition and the phenomenon the test is intended to examine.

Target

A gene, region, variant, microorganism or biomarker that a molecular test is designed to detect or measure.

Confirmatory test

A subsequent test or assessment used to confirm a screening result or analytical signal before assigning diagnostic or clinical meaning.

Tumour-agnostic

An approach that considers relevant molecular alterations independently of the tumour's anatomical or histological type, according to specific indications and criteria.

Variant calling

The process of identifying genetic variants from sequencing data.

VUS — Variant of Uncertain Significance

A genetic variant whose significance is uncertain. It requires cautious interpretation because available evidence does not support a definitive pathogenic or benign classification.

Integrated workflow

An analytical pathway combining different technologies at complementary stages, such as NGS for broad profiling and PCR for targeted measurement of selected targets.

Specialist workflow

An analytical pathway designed for a complex or non-standard question, which may require custom panels, dedicated bioinformatics, specific controls and outputs adapted to the setting.

Appendix C

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