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Review Article

Challenges in Y-DNA Recovery from Fabric: Effects of Environmental Degradation and Implications for Forensic Casework

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Abstract

Y-chromosomal DNA (Y-DNA) testing plays a critical role in forensic investigations involving male suspects, especially when traditional autosomal DNA evidence is insufficient or degraded. This review explores how different environmental factors—such as heat, moisture, Ultraviolet (UV) exposure, and microbial activity—impact the ability to recover Y-DNA from fabrics commonly found at crime scenes, including cotton, polyester, and denim. The study found that longer exposure to harsh environments, especially humidity and UV radiation, led to a sharp drop in the amount and quality of recoverable Y-DNA. The type of fabric also influenced results, with cotton generally retaining more DNA than synthetic materials like polyester. These findings reinforce the need for quick evidence collection and proper storage to preserve the integrity of Y-DNA. Several real-world cases are highlighted where Y-DNA analysis provided clear forensic outcomes, especially when autosomal DNA failed due to issues like allelic dropout—where one or more genetic markers fail to appear during testing—or secondary transfer, which occurs when DNA is unintentionally passed from one surface or person to another. In such cases, Y-DNA profiling was crucial in narrowing down or identifying male suspects, particularly when other forms of DNA were inconclusive. This review underscores the unique value of Y-DNA analysis in situations involving degraded or limited biological material and calls for the development of better recovery techniques to improve success in challenging forensic contexts.

Introduction

Y-chromosomal DNA (Y-DNA) has become an essential tool in forensic science, particularly in sexual assault cases where identifying male perpetrators is crucial [1]. Unlike autosomal DNA, which is inherited from both parents and recombines each generation, Y-DNA is passed down nearly unchanged from father to son, making it highly effective for tracing paternal lineage and identifying male contributors in mixed or degraded samples [1]. This male-specific inheritance allows forensic analysts to establish direct links between biological evidence and potential male suspects, especially when autosomal DNA analysis is inconclusive [1]. In addition to criminal investigations, Y-DNA is widely used in paternity testing, identifying victims in mass disasters, and resolving historical cases involving unidentified remains [2]. However, extracting high-quality Y-DNA from physical evidence—especially from semen-stained fabrics like clothing or bedding—remains challenging due to variables such as fabric type and environmental exposure [3]. Natural fabrics like cotton tend to retain DNA more effectively because they are porous and absorbent, while synthetic fabrics such as polyester and nylon resist DNA retention due to their smooth, water-repelling surfaces [4]. Environmental conditions further complicate DNA recovery. High temperatures (above 50°C) can fragment DNA by breaking down protective proteins [6], while soil introduces microbes and pH changes that chemically degrade sperm cells and genetic material [7]. Laundering or water exposure may physically wash away or damage DNA, significantly reducing the chances of successful extraction [8]. Microbial contamination, especially in biological mixtures like semen combined with vaginal fluids, accelerates DNA breakdown through enzymatic activity [7]. These stressors can lead to allelic dropout, where certain genetic markers

fail to amplify during analysis, resulting in incomplete profiles, and secondary transfer, where DNA unintentionally moves between surfaces, complicating interpretation. While autosomal DNA recovery has been extensively studied, there is limited research on how fabric type, time, and combined environmental stressors affect Y-DNA integrity. Most studies examine isolated factors like humidity or heat, with few exploring interactions between multiple conditions such as burial, microbial activity, and washing [7,9]. Recovery of Y-DNA from aged, degraded, or low-level samples remains an area in need of more research. To improve Y-DNA reliability in forensic contexts, there is a growing need for advanced extraction techniques, more sensitive amplification methods, and preservation strategies tailored to specific fabric types [10]. Technologies like Next-Generation Sequencing (NGS) and Y-STR (short tandem repeat) profiling have significantly enhanced the detection and resolution of Y-DNA. However, future studies should address overlooked variables such as fabric porosity, exposure duration, and microbial diversity to optimize DNA recovery. Developing standardized methods for extracting Y-DNA from synthetic or blended fabrics—particularly in cases involving semen stains—is vital for maximizing its forensic usefulness [11,12]. The value of Y-DNA analysis has been proven in real-world cases. In the Boston Strangler investigation, Y-DNA testing linked crime scene evidence to a living relative of the main suspect, Albert DeSalvo, confirming his role decades after the murders [13]. Similarly, in the Vaatstra case in the Netherlands, autosomal DNA failed to identify a suspect, but mass Y-DNA screening pointed to a male relative of the perpetrator, ultimately leading to a confession and conviction [14,15]. These cases highlight the strengths of Y-DNA in resolving forensic uncertainties where autosomal methods fall short, especially in male-specific or degraded samples. Y-STR analysis has proven especially valuable in solving cold or previously unsolved cases, particularly when the evidence contains a mixture of male and female DNA. In situations where the female is high, Y-STRs help isolate and identify the male component, making it easier to confirm a suspect's involvement. This type of testing can establish stronger connections between a suspect and the crime scene. When combined with other forensic tools like investigative genetic genealogy, Y-STRs have become an increasingly effective method for uncovering the truth in complex, long-standing cases [16,17].

Y-DNA

The Y chromosome, along with the X chromosome, forms one of the two human sex chromosomes. In the human cell nucleus, these sex chromosomes exist alongside 22 pairs of autosomal (non-sex) chromosomes. Typically, the presence of one X and one Y chromosome results in male characteristics, while two X chromosomes usually lead to female characteristics. However, certain genetic mutations or rare changes in chromosome number can affect this biological outcome [18]. Some regions of the Y chromosome can exchange genetic material with the X chromosome. However, the remaining portion, known as the male-specific region (MSY), contains approximately 70 genes that are unique to males and do not undergo genetic exchange. One of the important genetic markers found on the Y chromosome is the Amelogenin (AMEL) gene. In forensic analysis, scientists often use this marker to differentiate between male and female DNA. AMELY is located on the Y chromosome, while AMELX is located on the X chromosome [19]. Since the Y chromosome is passed almost unchanged from father to son, Y-chromosome STR (short tandem repeat) markers are widely used in forensic investigations to trace male lineage [1]. These STR markers are unique to the Y chromosome and have become essential tools in identifying male contributors in DNA samples [19]. For example, markers like DYS391, located on the long arm of the Y chromosome, have been added to standard autosomal DNA testing kits. This specific marker helps determine an individual's gender and can identify male relatives, making it particularly valuable in forensic casework [20,21].

Factors affecting the Y-DNA recovery

Fabric composition: Fibres used in forensic investigations are typically classified into two main categories: natural fibres, which are derived from plant or animal sources, and synthetic fibres, which are produced through chemical processes. Plant-based fibres such as cotton are primarily composed of cellulose, a polysaccharide made up of β-D-glucose units. In contrast, animal-based fibres like wool, hair, and silk are protein-based, consisting of amino acid chains such as keratin or fibroin [22]. Natural cellulose fibres like cotton, along with regenerated fibres such as rayon, contain abundant hydroxyl (–OH) groups. These polar functional groups allow for hydrogen bonding and dipole interactions with nucleic acids, enhancing DNA adherence and improving its stability on these fibres. On the other hand, synthetic fibres such as polyester—which is produced through condensation polymerisation of aromatic dicarboxylic acids and diols—and acrylic fibres, derived from polyacrylonitrile ($–CH_2–CHCN–$), are less favourable for DNA binding. Their relatively hydrophobic surfaces and lack of polar functional groups reduce their capacity to retain DNA [23,24]. In forensic investigations—particularly in cases involving body fluids such as blood, semen, or saliva deposited on fabrics—the interaction between DNA and the fibre material becomes crucial. Cotton, due to its porous structure and abundance of hydroxyl groups, promotes strong hydrogen bonding with DNA molecules. This characteristic enhances DNA retention and recovery, making downstream amplification more reliable. However, extended exposure to environmental conditions can degrade these interactions over time [25]. In contrast, synthetic fabrics like polyester and nylon—commonly found in hosiery and bedding—have smooth, hydrophobic surfaces that tend to repel biological fluids. As a result, these materials exhibit limited DNA retention due to weaker dipole–dipole interactions and poor absorption characteristics, which complicates forensic

DNA recovery [26]. Additionally, the tightly woven structure and low surface energy of hosiery fabrics further hinder the penetration and retention of nucleic acids. Given these challenges, it is essential to develop and refine DNA extraction techniques tailored to specific fabric types, especially in forensic contexts where biological samples may be degraded, limited, or contaminated [24,25].

Environmental factors

Temperature: Temperature plays a crucial role in affecting both the physical structure of fabrics and the stability of biological materials deposited on them, such as blood or semen. At low to moderate temperatures (4 °C to 37 °C), there are minimal visible or chemical changes to the fabric or the biological stains. Under these conditions, DNA remains relatively intact, allowing for efficient extraction and successful forensic analysis, as the cellular and molecular structures are well-preserved [27]. However, when temperatures rise to between 50 °C and 100 °C, biochemical changes begin to occur in the biological stains. For example, blood may darken due to oxidative reactions, such as the transformation of haemoglobin into methaemoglobin. These chemical alterations are often accompanied by the thermal denaturation of proteins, which normally help protect and stabilise DNA molecules. As these protective proteins break down, the DNA becomes more vulnerable to fragmentation and degradation, resulting in lower recovery rates and diminished quality of the genetic profile [28]. The most severe damage occurs when biological evidence is exposed to extreme heat or direct flames, as seen in fire-damaged fabrics. In such cases, both the fabric and biological material may become charred or entirely carbonised, making DNA either highly degraded or completely unrecoverable. High-temperature exposure causes extensive DNA strand breaks, and the presence of soot or combustion byproducts can interfere with critical downstream processes like Polymerase Chain Reaction (PCR) amplification [29]. These challenges significantly reduce the forensic value of the evidence. Studies by Abdel Hady, et al. (2021) and Karni, et al. (2013) highlight the temperature thresholds at which DNA degradation becomes so extensive that it is no longer suitable for forensic analysis. Their findings show that while moderate heat exposure may still allow for partial DNA recovery, exposure to the high temperatures typical of combustion generally causes irreversible molecular damage, ultimately compromising the reliability of forensic results [30] (Table 1).

Table 1: DNA recovery at different temperatures [30].

Temperature Condition	Mean DNA Concentration (ng/ml)	Effect on Y-DNA Recovery	Explanation
Positive Control (Room Temp)	90.90 ± 2.85	High (Optimal recovery)	No degradation; ideal conditions for DNA preservation.
4 °C	89.55 ± 4.11	High	Cold preserves DNA integrity and structure
20 °C	89.30 ± 4.23	High	Like control, no significant impact.
37 °C	87.05 ± 9.87	Moderate to High	Mild reduction but not significant; DNA is still stable
50 °C	65.06 ± 13.75	Moderate to Low	Noticeable degradation of DNA due to protein denaturation.
100 °C	59.85 ± 13.82	Low	Significant degradation of proteins and DNA strands.
Burn	13.65 ± 3.95	Very Low (Minimal or no recovery	Complete charring and soot formation interfere with DNA extraction.

Soil

Soil plays a significant role in the preservation and recovery of Y-chromosomal DNA (Y-DNA) from semen stains, particularly when fabrics have been buried or are in prolonged contact with the ground. Various environmental factors within the soil—such as moisture levels, pH, microbial activity, and the presence of organic compounds—contribute to the degradation of sperm cells and their genetic material [31]. Moisture is especially important for maintaining DNA stability. Extremely dry soil conditions can desiccate semen samples and concentrate harmful substances like endocrine-disrupting chemicals, which damage both sperm structure and DNA integrity. On the other hand, too much moisture encourages microbial growth, which accelerates DNA breakdown. Research suggests that low moisture levels in soil cause oxidative stress and molecular instability in sperm cells, reducing the likelihood of obtaining a viable Y-DNA profile [32]. Soil pH is another crucial factor. Acidic environments (low pH) negatively affect sperm viability and speed up the deterioration of cellular structures that are essential for DNA extraction. Studies have shown that DNA in semen breaks down more rapidly in acidic soils compared to neutral or alkaline soils, largely due to increased hydrolysis and damage to sperm membranes [33]. Additionally, humic substances—complex organic compounds commonly found in soil—can bind to DNA and interfere with enzymatic reactions in the lab, such as Polymerase Chain Reaction (PCR) amplification. This interference can reduce the accuracy of forensic tests and increase the risk of false negatives [34]. The type of fabric involved also impacts semen preservation in buried samples. Synthetic fibres like polyester, made through the polymerisation of ethylene glycol and terephthalic acid, have tightly packed molecular structures with low porosity (10–30 nm). These characteristics reduce fluid absorption and speed up drying, which can cause sperm degradation within the first 24 hours after burial [31]. In contrast, natural fibres like cotton offer larger pore sizes and higher absorbency, creating a more protective environment for biological material, including semen. Ultimately, the success of Y-DNA recovery from buried evidence depends on the complex interaction between soil chemistry, microbial communities, and the physical properties of the fabric. A thorough understanding of these factors is essential for improving DNA extraction protocols in forensic investigations involving outdoor or concealed crime scenes [35].

Microorganisms

The complex biological fluid known as human semen is made up of several different substances, such as N-acetyl-D-glucosamine (GlcNAc), acid phosphatase, citric acid, calcium, zinc, and fructose [36,37]. GlcNAc is a simple sugar that microbes use as a source of energy to support their growth. Due to its slightly alkaline pH and rich nutrient content, semen provides an optimal environment for microbial proliferation and biofilm formation, which in turn accelerates the degradation of biological material [38,39]. The forensic evidence from several sexual assault situations include a combination of vaginal fluids and semen. This combination, abundant in proteins, carbohydrates, and epithelial cells from both sources, further enhances microbial activity, thereby expediting DNA degradation. Vaginal fluid contains various proteins, including albumin, α1-antitrypsin, and α2-haptoglobin, and other secretions from glandular sources that support the growth and maintenance of microbial communities [40,41]. Biological stains like blood or other fluids on clothes can help microbes grow. Given these conditions, the rapid collection, storage, and examination of the biological evidence in sexual assault cases are important to minimise microbial contamination and preserve DNA integrity [42].

Laundering effect

Washing clothing after a sexual assault can greatly reduce the chances of detecting Y-chromosomal DNA (Y-DNA). Although laundering does not always eliminate all genetic material, it significantly diminishes the visibility and structural integrity of semen stains. Mechanical agitation, high water temperatures, and the use of detergents work together to break apart and disperse sperm cells [43]. This makes it much more difficult to extract and successfully amplify DNA. In some cases, small amounts of sperm cells may remain embedded in certain fabric types. However, repeated washing cycles lower the concentration of sperm cells, making it harder to obtain a complete Y-STR profile. Research has shown that absorbent fabrics like cotton can retain detectable levels of Y-DNA even after several washings [44]. For instance, Nolan and colleagues reported in 2018 that sperm cells were still detectable on cotton and terry cloth fabrics after six laundry cycles. However, the DNA profiles recovered from these samples were often partial or of reduced quality [45].

Water environment

Exposure of semen stains to water—whether from rain, immersion, or intentional rinsing—can significantly reduce the likelihood of successfully recovering Y-chromosomal DNA (Y-STR). Since Y-STR analysis is vital for identifying male DNA in forensic investigations, water exposure poses a serious challenge to obtaining reliable results. Contact with water can dilute or wash away sperm cells, which are the primary source of male-specific DNA. Prolonged exposure can damage the membranes of sperm cells, leading to fragmentation and degradation of the nuclear DNA [46]. These effects are worsened when environmental factors such as soil, wastewater, or biological debris are present, as they introduce nucleases and microbial activity that accelerate DNA breakdown [47]. The type of surface or fabric where semen is deposited plays a major role in DNA preservation after water exposure. Natural fabrics made from plant-based fibres like cotton generally have a loose weave and large pore sizes (approximately 50–100 nanometres), which allow for high fluid absorption. This absorbent quality enables semen to be trapped deeper within the fabric, shielding it from environmental exposure and washing. Therefore, limited water exposure—such as light rain or brief rinsing—does not always prevent successful Y-STR recovery, especially if the fabric is quickly dried and stored under proper conditions [48]. In contrast, synthetic fabrics like polyester and nylon, made from plastic-based polymers, feature smaller pore sizes (10–30 nanometres) and low absorbency. These materials tend to retain semen on the surface, making sperm cells more vulnerable to being washed away during water contact. As a result, Y-STR recovery from synthetic textiles after water exposure is generally poor [49]. Semi-synthetic fabrics, such as those containing rayon or spandex, also have low absorbency and tightly woven structures like synthetic materials. However, due to their elasticity and close-fitting use, these fabrics may spread semen more widely across the surface, increasing sperm exposure to environmental damage. In such cases, Y-DNA recovery is typically low unless the stains are fresh and processed promptly [50].

Fabric Type	Water Absorption	Sperm Retention	Y-DNA Recovery After Water Exposure
Natural fabric	High	High (deep pores)	Moderate to Good
Semi-synthetic fabric	Moderate	Moderate (thin layer)	Poor to Moderate
Synthetic fabric	Low	Low (surface only)	Poor

Forensic importance

Sexual assault cases: The Y chromosome plays a vital role in forensic DNA analysis, particularly in resolving mixed DNA profiles involving both male and female contributors, as well as in cases of oligospermia or azoospermia [51]. Traditionally, forensic scientists have relied on autosomal Short Tandem Repeat (STR) markers due to their high polymorphism and ease of interpretation. Commercially available STR kits, such as PowerPlex® 16, AmpFlSTR® SGM Plus™, and AmpFlSTR® Profiler Plus™, are commonly used for this purpose. These kits also target the amelogenin gene, which allows for determining the biological sex of the DNA donor [52]. However, despite their advantages, traditional autosomal STR kits have limitations when it comes to distinguishing male DNA in mixed samples or in cases where male DNA is present in very low quantities. To overcome these challenges, Y-chromosome STR (Y-STR) typing was introduced, enabling the detection of male-specific genetic profiles. Early-generation kits like PowerPlex® Y

included 12 Y-STR markers. Later advancements, such as the AmpFlSTR® Filer™ and PowerPlex® Y23 kits, expanded this to 17 and 23 loci, respectively [53,54]. One of the key limitations of Y-STRs is their relatively low mutation rate—approximately 1×10^3 mutations per locus per generation. This limited variability means that closely related male individuals, such as paternal relatives, may share identical Y-STR haplotypes. As a result, the ability to distinguish between them is reduced, which may increase the risk of false exclusions. Additionally, the informativeness of Y-STR markers can vary significantly across different populations, highlighting the importance of using population-specific reference databases when interpreting Y-STR profiles in forensic investigations [54,55].

Paternity testing

In paternity testing, the comparison of specific Short Tandem Repeat (STR) loci helps determine biological relationships. If multiple mismatches are found between an alleged father and the child at these loci, the individual is typically excluded as the biological parent [56]. In the past, methods such as Polymerase Chain Reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis were commonly used. However, these techniques have become largely outdated due to their limited sensitivity and lengthy processing times, especially with degraded DNA samples [57]. Consequently, Y-chromosomal STR (Y-STR) analysis has gained prominence in both relationship testing and forensic identification. Its strength lies in its ability to generate male-specific genetic profiles, even from highly degraded biological material. Y-STR profiling involves the amplification of male-specific genetic markers, followed by fragment separation via capillary electrophoresis and allele designation through genotyping software [58]. This approach has proven highly effective in a variety of forensic applications, including identifying victims of armed conflicts, mass disasters, and historical events [58,59]. A notable early use of Y-STR analysis was in 1997 by Daniel Coach and colleagues in Argentina. They successfully identified eight missing individuals among 340 skeletal remains recovered from a mass grave. The team used eight Y-specific STR loci—DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393—and compared the profiles to those of living paternal relatives [60]. Similarly, following the 2012 Tazreen Fashions factory fire in Bangladesh, which claimed 112 lives, Y-STR analysis was critical for victim identification. Of the 59 severely burned bodies recovered, 43 were matched with their relatives using a combined autosomal and Y-STR approach. Likelihood ratios between unknown samples and reference profiles were calculated using Geno Proof software v2.0 (Quality GmbH, Germany), enabling successful identifications [58]. Y-STR technology has also been instrumental in post-conflict identifications. In 2007, researchers analysed skeletal remains from a World War II mass grave in the Dalmatian Mountains. The initial analysis was done using the PowerPlex® Y System (12 loci), followed by confirmation with the AmpFlSTR® Filer™ kit (17 loci). Three individuals were positively identified, with paternity probabilities calculated at 8.6×10^8, 1.36×10^{13}, and 8.6×10^{11}, respectively [60]. In a related 2009 study, six individuals were identified from a group of eleven exhumed from a World War II mass grave in the Kočinski Rog region of Slovenia using a combined Y-STR and Mini-STR strategy. Additionally, two more individuals recovered from single graves in the Ljubljana area were identified solely through Y-STR profiling. The analysis used the PowerPlex® Y System for amplification, capillary electrophoresis via the ABI PRISM 310 Genetic Analyser, and match probability estimation through DNA•VIEW™ software [59]. Together, these cases highlight the strength and reliability of Y-STR analysis in challenging forensic situations, particularly when working with degraded remains or in mass casualty incidents. The ability to trace male lineage from minimal or compromised DNA makes Y-STR profiling a cornerstone in modern forensic genetics [58].

Genealogical studies

The Y chromosome's unique pattern of inheritance makes it highly valuable in both forensic science and population genetics. Unlike autosomal and X chromosomes, over 95% of the Y chromosome is non-recombining, meaning it is passed from father to son with minimal changes across generations [61]. This non-recombining region accumulates stable mutations—such as single-nucleotide polymorphisms (SNPs)—that remain linked over time, forming distinct haplotypes. When individuals share the same combination of these mutations, they are grouped into haplogroups, which represent specific paternal lineages [62]. By examining both Y-STRs and Y-SNPs, researchers can reconstruct paternal family trees, classify population groups, and trace historical migration routes. These analyses have provided strong genetic evidence supporting the "Out of Africa" theory, with African populations displaying the greatest diversity in Y-chromosome lineages, indicative of a longer evolutionary timeline [63]. In addition to advancing anthropological knowledge, these insights have enhanced forensic applications. Y-chromosome markers have been used to estimate the biogeographical ancestry of unidentified male individuals in criminal investigations, particularly in cases involving degraded or limited DNA samples. In recent years, Next Generation Sequencing (NGS)—especially through Massively Parallel Sequencing (MPS)—has revolutionised forensic genomics. MPS allows for the simultaneous sequencing of multiple genetic loci, providing high-resolution data that improves individual identification and the resolution of DNA mixtures [64,65]. Although MPS has primarily been applied to autosomal STR analysis, there is increasing interest in its use for Y-STRs and Y-SNPs. Studies indicate that MPS-based Y-chromosome profiling offers greater discriminatory power and finer resolution of complex haplotypes compared to traditional capillary electrophoresis methods [66]. However, despite its promise, the forensic potential of Y-chromosome MPS remains underutilized compared to autosomal

sequencing. Ongoing research into sequencing Y-STRs and Y-SNPs could substantially improve the precision of paternal lineage tracing, support human identification in mass disaster scenarios, and enhance ancestry inference techniques in forensic investigations [67].

Conclusion

Y-chromosomal DNA (Y-DNA) remains a vital element in forensic genetics, particularly for identifying male contributors in complex or degraded biological samples. Its importance is especially pronounced in mixed DNA profiles and cases involving minimal or compromised material. The efficiency of Y-DNA recovery is influenced by several factors, including environmental exposure and the type of substrate on which the biological material is deposited. Recent technological advancements—particularly the combined use of Y-chromosome short tandem repeats (Y-STRs) and single-nucleotide polymorphisms (Y-SNPs) alongside Next Generation Sequencing (NGS)—have significantly enhanced the resolution of Y-DNA analysis. These improvements not only increase the ability to distinguish between closely related male individuals but also support studies in human ancestry and population genetics, expanding both forensic and anthropological applications. Recovering Y-DNA from aged, low-quantity, or environmentally degraded samples—such as those exposed to burial or water—remains a considerable challenge. Nevertheless, the development of optimised DNA extraction methods, improved amplification chemistries, and fabric-specific preservation strategies shows promise in increasing the success rate of Y-DNA profiling under such difficult conditions. Moreover, the integration of Massively Parallel Sequencing (MPS) technologies provides new opportunities for analysing highly fragmented Y-DNA, thereby broadening its applicability in forensic and genealogical investigations. Although challenges remain, the role of Y-DNA in forensic science is poised to grow, with ongoing research focused on overcoming the limitations posed by environmental conditions and substrate types.

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