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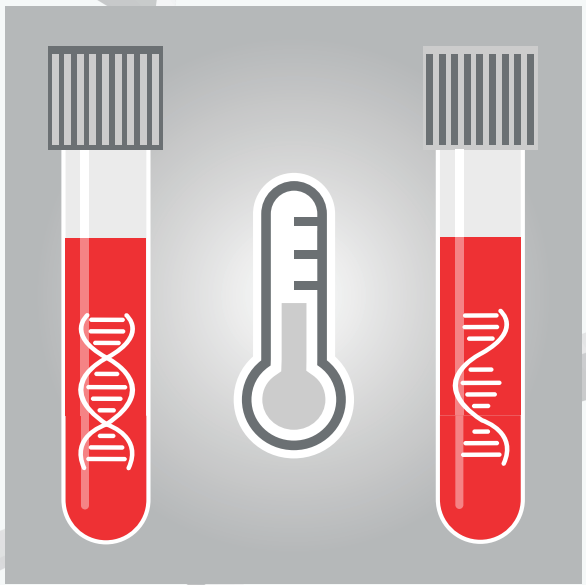

THINKING OUTSIDE THE FREEZER:

DNA AND RNA STORAGE

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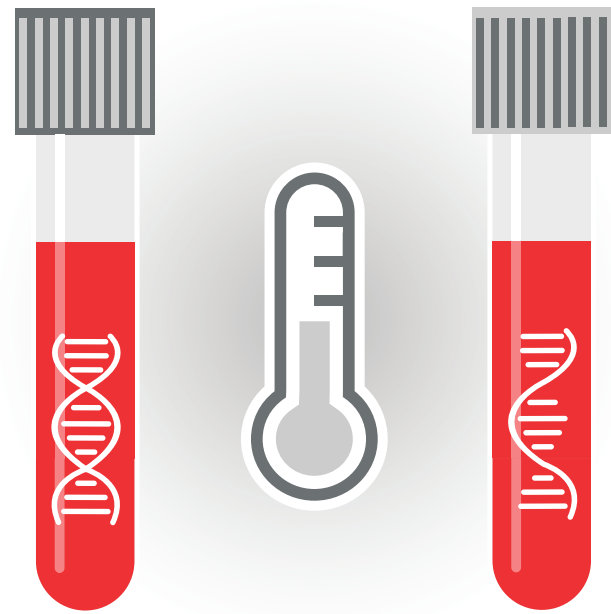
INTRODUCTION

While ultra-low temperatures are the current state-of-the-art solution for biospecimen storage, ambient storage is an interesting option and could be superior in certain limited situations. For many types of samples it's possible that we will always have to use ultra-low temperatures for long-term storage. For other sample types, such as DNA and RNA, there are alternative solutions.

Precision medicine relies on the ability to accurately sequence and interpret information stored in the DNA and RNA from a given individual. As the need to understand the underlying genetic background for patients and clinical trial participants has increased, so too has the need to collect high-quality DNA and RNA samples. Next generation sequencing (NGS) and RNA-seq based studies are the primary tools utilized to obtain this data and these technologies rely on high-quality samples.

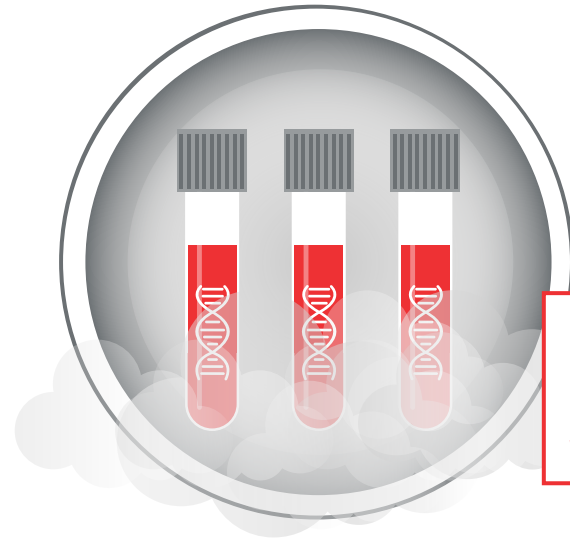
There are alternative solutions for DNA & RNA storage options



STORAGE OF DNA

Collection and storage of samples that maintain nucleic acid integrity is expensive, especially when the collection sites are in remote areas. Although storage of DNA at -20°C and -80°C remains a common method for storing extracted DNA samples, there have been multiple efforts to develop methods to store purified DNA samples at room temperature.

Multiple freeze-thaw cycles can lead to shearing and subsequent DNA degradation. One solution is to store DNA in liquid nitrogen in the vitreous state. Another solution is to store DNA in a dried, ambient temperature. In terms of stability, the benefit of dried DNA is that dehydration also removes the water that participates in the hydrolytic reactions that lead to strand breakage in both DNA and RNA (Kansagara, McMahon et al., 2008).



One solution is to store DNA in liquid nitrogen in the vitreous state.

Another solution is to store DNA in a dried, ambient temperature.

STORAGE OF DNA

Storage of DNA at ambient temperature without any form of stabilization can lead to degradation for a multitude of reasons, including contamination of cellular nucleases depending on the form of the sample. To combat this, groups have used several methods of drying samples to include:

- spray drying,
- spray freeze drying,
- air drying,
- lyophilization,
- and drying with the addition of commercially available stabilizing compounds.

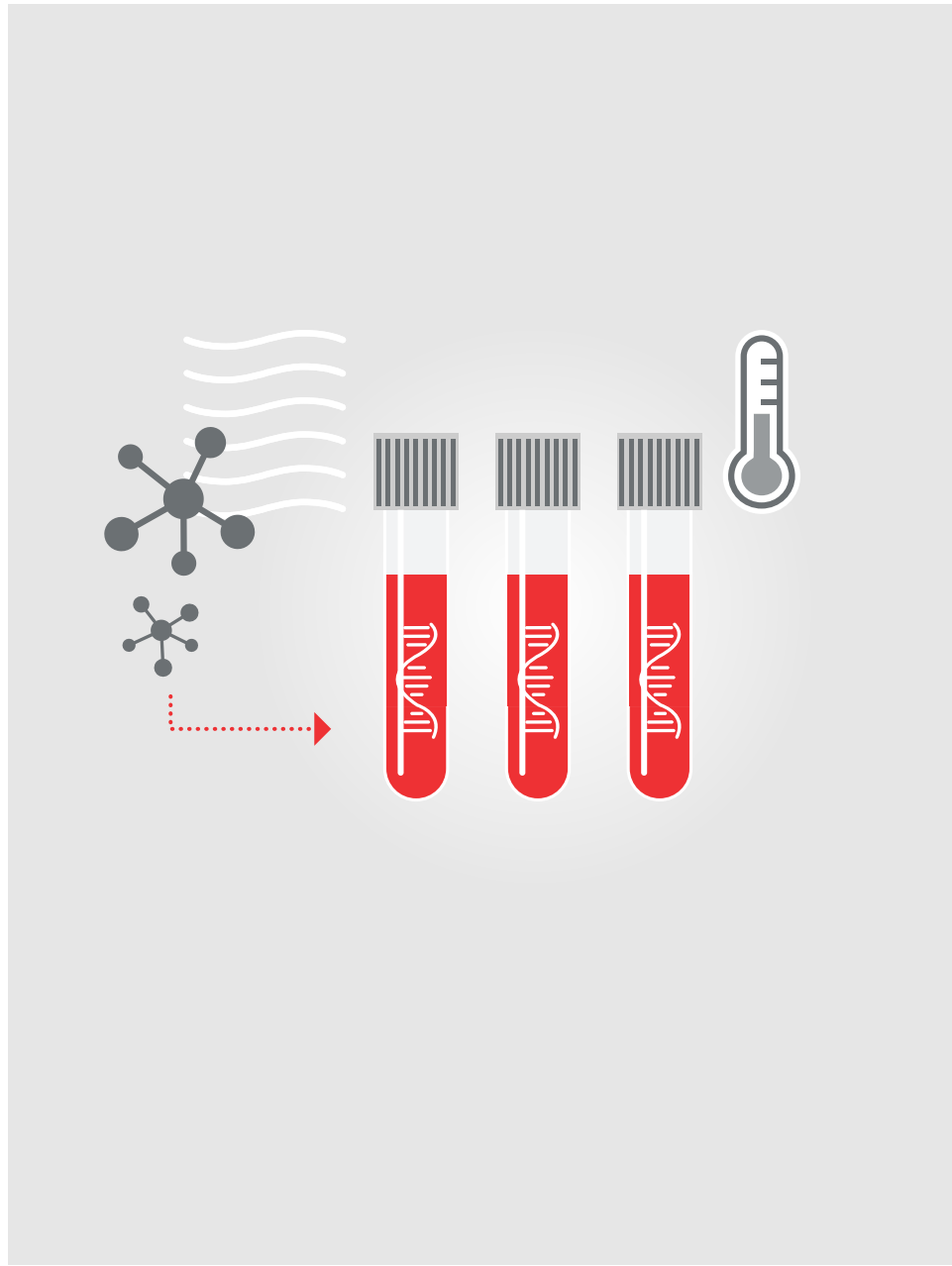
Other options for DNA stabilization include the usage of FTA Cards and the precipitation of DNA in ethanol. However, these options can be time-consuming to extract the DNA for downstream purposes and are not suitable for all applications, especially in high throughput labs and clinical settings. A relatively new and promising approach is to use commercially available stabilizers. Adding a stabilizer to wet DNA allows samples to be dried down and stored at ambient temperatures without the risk of degradation. This allows for easier and more economical sample storage in resource challenged settings, particularly the many clinical trials that are conducted in locations with little infrastructure for proper sample processing and storage.

STORAGE OF RNA

Similar to DNA, RNA quality is influenced by freeze/thaw cycles and cellular stress responses, as well as the tissue processing protocols and storage conditions that occur prior to RNA extraction. Formalin-fixed paraffin-embedding (FFPE) is still the primary method of tissue preservation in pathology labs, but it makes RNA extraction difficult due to fragmentation. Flash freezing provides higher quality RNA than FFPE, but is often impractical for labs and clinical sites that have limited access to the required freezing facilities and limits sample collection to a centralized location.

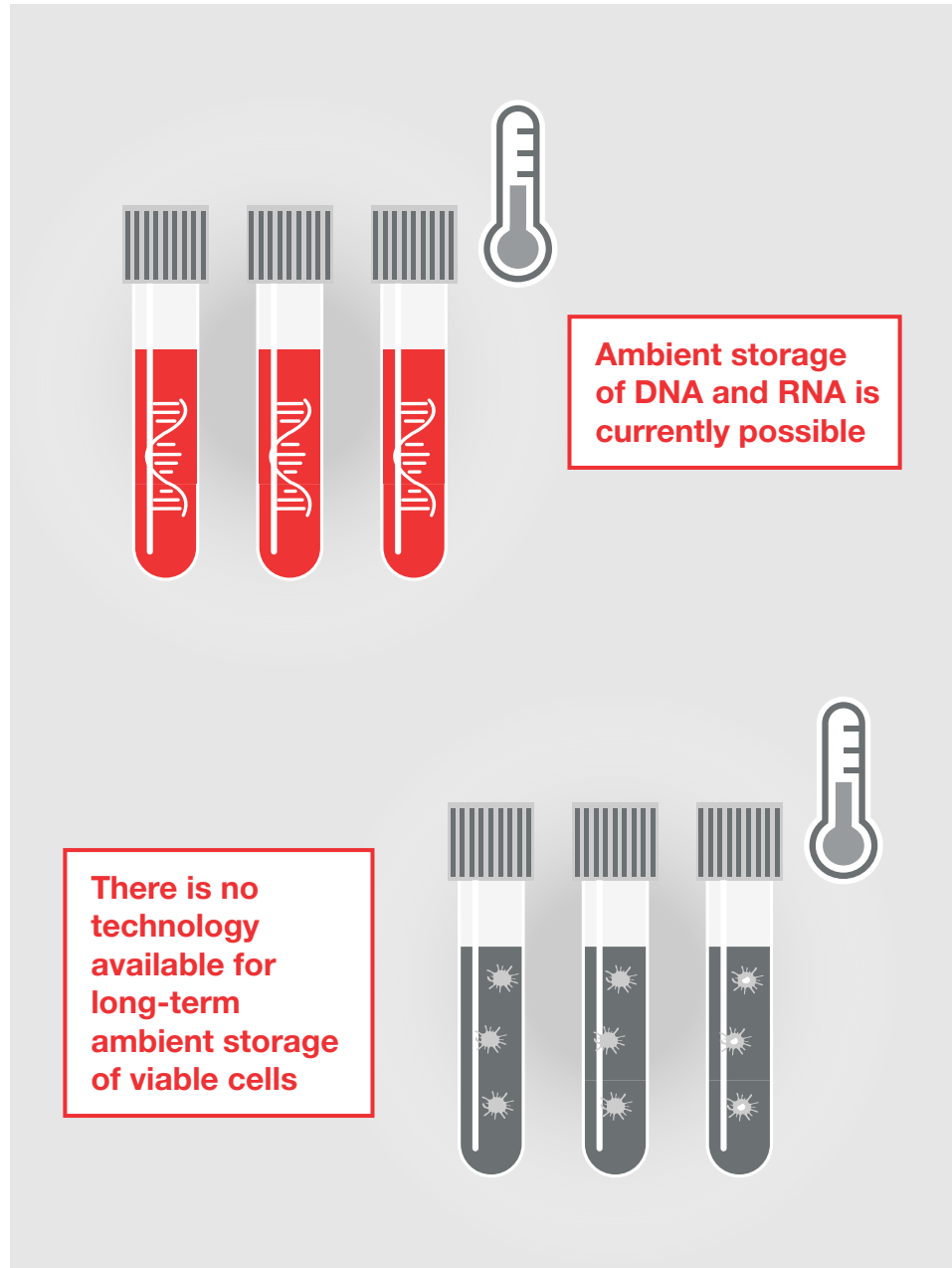
Commercial compounds, such as RNAlaterTM, allow small tissue pieces to be immediately submerged in the stabilizing solution without the need to freeze samples. These preserved tissue samples can be stored at room temperature for up to one week, 4°C for one month, and -80°C for longer periods up to six months before e xtraction. This solution precludes the need for specialized preservation equipment and provides collection sites the ability to isolate the RNA at a later time without jeopardizing the quality or quantity of RNA obtained, thus eliminating the need to immediately process tissue samples (Salehi, 2014).

Many clinical trials also collect and store cells for live-cell assays. The gold-standard for long-term cell storage is to maintain them in liquid nitrogen, at approximately -180°C. The cells are stored with a cryopreservant, typically DMSO, which helps reduce formation of damaging crystals, which can disrupt cellular membranes. When properly preserved and stored, cells can be revived and used successfully in assays decades later.



THE AMBIENT STORAGE OPTION

Although ambient storage of DNA and RNA is currently possible, storage of cells at ambient temperatures is a far more challenging problem. While there is no technology available for long-term ambient storage of viable cells, there have been some advancements with short-term storage. Since the timeframe for viability with these techniques is relatively short, the focus is currently on ambient transport of cell cultures. Typically, cells are collected or cultured, then embedded in some form of gel-like matrix. Some strategies involve mixes of sugars (Stefansson, Adams et al. 2016; Stefansson, Han et al. 2017), alginate hydrogels (Stefansson, Han et al. 2017), or mucin-like structures (Canton, Warren et al. 2016). Despite the fact that ambient long-term storage of viable cells won't be available for quite a long time, if at all, solutions exist today for DNA and RNA storage.



CONCLUSION

Here at Thermo Fisher Scientific, we understand the various needs of our clients and are capable of meeting those needs through our ability to handle material at temperatures ranging from ambient to cryogenic. Our teams of experts have a deep understanding of the complex logistics required to move samples between sites, store them properly to maintain their integrity, and manage an inventory to ensure that all samples are readily available for testing. We recognize that sample integrity is essential to our client's research and develop flexible solutions so they are able to accomplish their downstream goals. For example, the AstraZeneca UK Biobank offers long-term storage of, and rapid access to, approximately 1 million human biological samples from AZ Oncology clinical trials, collaborators and commercial sources.

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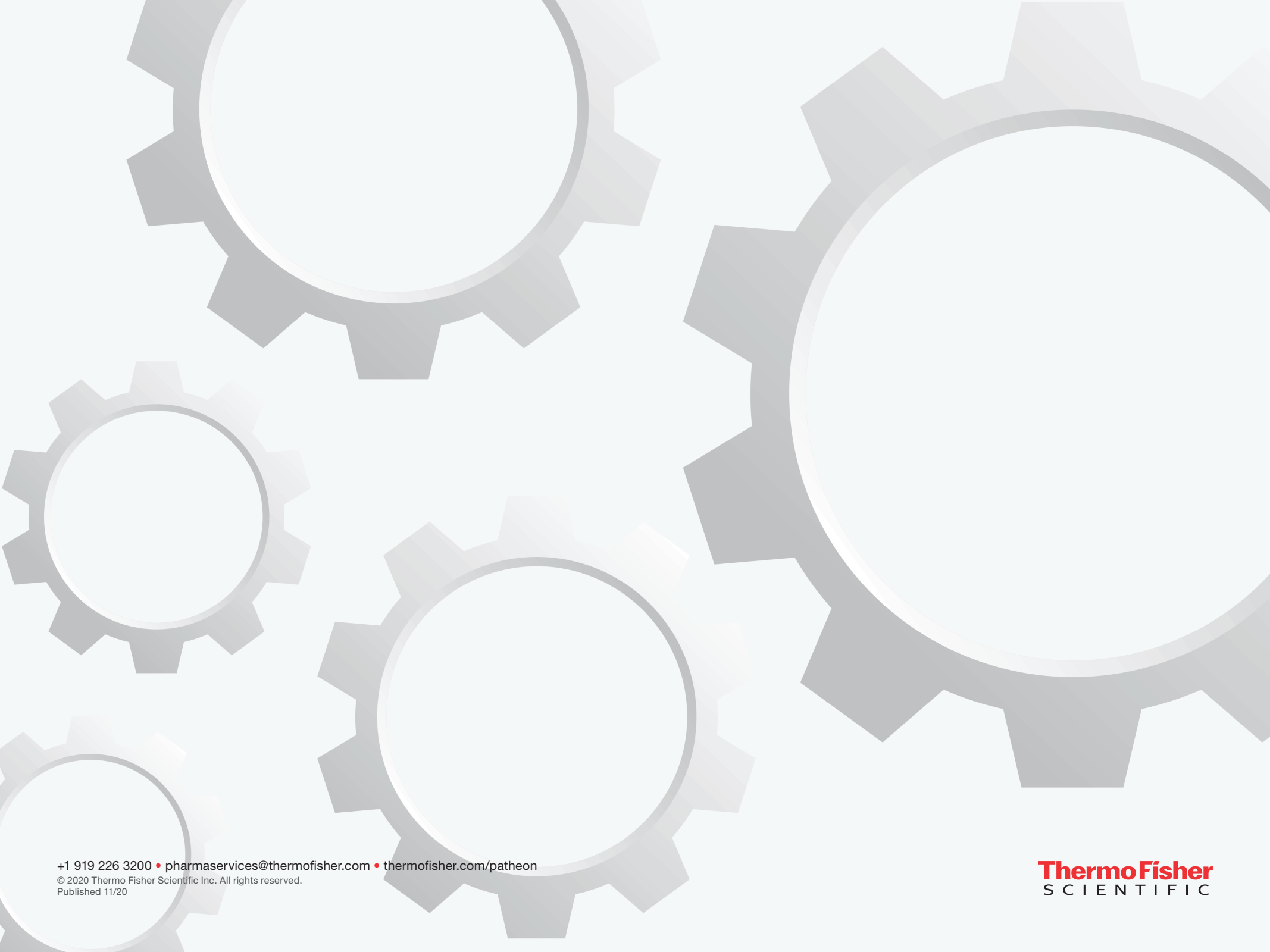

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