

Cell line development and utilisation trends in the cultivated meat industry

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Executive Summary

ALLEVIATING CELL LINE BOTTLENECKS TO FEED A GROWING WORLD

The cultivated meat industry aims to feed a growing global population and reduce the negative impacts associated with conventional animal meat production by manufacturing real meat from cultivated animal cells. While the industry has rapidly expanded over the past ten years, there remains a lack of publicly available information needed for forecasting and future planning. Specifically, more information is needed regarding the cell lines that companies are currently using and plan to use in the future. We need to identify actionable opportunities to increase the accessibility of cell lines, optimise their characteristics, and enhance their suitability to the cultivated meat production process, so as to create superior quality products at a large scale and lower cost. The data presented in this report is intended to supplement GFI's existing efforts to alleviate bottlenecks related to cell line accessibility, such as our [cell line database](#).

MAPPING INDUSTRY TRENDS WITH REAL-WORLD DATA

A variety of cell lines and cell types serve as building blocks for cultivated meat production. To better understand how these are being used and developed by the cultivated meat industry, we sent a questionnaire to companies using cell lines for the research and development (R&D) of cultivated meat products or those supplying the industry with cell lines and other process inputs. Based on the responses to this survey, we have distilled a snapshot of this emerging industry's current progress and needs, with an eye towards future cell line demands, characterisation requirements, and regulatory considerations.

SURVEYING THE CULTIVATED MEAT INDUSTRY

In November 2022, a questionnaire was sent to a pool of companies ranging from early-stage startups to more established entities. A total of 44 companies responded to our survey, and a majority of them had been in operation for less than two years at the time of surveying ($n = 23$; 52%). For context, out of 156 companies listed in GFI's [company database](#), 67 (43%) had been in operation for less than two years as of 2022. Therefore, we believe that the results presented skew towards newer companies, owing to a higher likelihood of requiring support with cell lines and a greater willingness to communicate their needs.

KEY FINDINGS AND TAKEAWAYS

This study elucidated a number of common challenges and bottlenecks companies are facing in obtaining commercially relevant cell lines. Of concern, there is almost certainly significant duplication of effort with similar species, cell types, and product characteristics being pursued by many companies, and cell line development largely being conducted through resource-intensive in-house efforts. A limited understanding of regulatory and religious requirements was also a common challenge identified by respondents, with a lack of clarity around acceptable cell line derivation and safety testing documentation for various jurisdictions. The following are summarised key findings of the report.

Species selection: A majority of companies are interested in obtaining cell lines from both terrestrial and aquatic species. Cow, pig, sheep, and lamb are the highest-priority terrestrial species, and salmon, tuna, and other finfish are the highest-priority aquatic species of interest.

Desired cell types and accessibility: Myoblasts, fibroblasts, and mesenchymal stem cells (MSCs) were the most used cell types by companies. This seems to be driven, at least in part, by a lack of access to other, potentially more suitable, cell types. For terrestrial species, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) were highlighted as the most difficult to source. In addition to ESCs and iPSCs, myoblasts also ranked highly as a difficult-to-source cell type for aquatic species. Therefore, cell line providers who are able to offer cell

types that are hardest to source would be highly sought-after. This is also supported by the finding that almost half of the companies are open to purchasing off-the-shelf cell lines.

Cell sourcing: There is a heavy reliance on slaughtered animals for sourcing cells. Inadequate access to live animals and low proximity to cell isolation facilities represent key barriers for companies to access cell lines, particularly for those looking to move away from slaughtered animal sources. This represents an opportunity for enterprising livestock and seafood producers, as well as farmers and ranchers, to partner with companies and create a novel revenue stream for their operation.

Cell line characteristics: Overall, companies expressed a hesitancy with using genetically engineered or modified cell lines, but are keen to use spontaneously immortalised cells that may not qualify as genetically modified based on regulatory definitions in many jurisdictions. In addition to seeking cell lines with high proliferative potential and genetic stability, well-characterised cell lines with the ability to grow in suspension were highly desired. Therefore, there is a critical need for cell line providers who can tailor their offerings to match these most highly desired traits. Successful suspension adaptation has only been achieved by less than half of the surveyed companies through their own internal R&D efforts. In contrast, a majority of the surveyed companies confirmed successful adaptation of at least one cell line to grow in serum-free conditions, highlighting that serum-free growth is no longer a bottleneck.

Regulatory hurdles: Most companies had low confidence in their understanding of the documentation and safety testing requirements to obtain regulatory approval of cell lines in high-priority markets, such as the United States (U.S.) and Singapore. This highlights a need for greater accessibility and approachability to regulatory bodies and experts to provide more clarity on process and testing requirements. Regulators should consider tailoring regulatory guidelines to answer the knowledge gaps that companies have, such that companies are able to comprehend these guidelines and adhere to them easily.

Religious certifications: A strong preference was shown by companies to generate cell lines that could be certified as halal and/or kosher. However, the absence of resources that adequately explain how cultivated meat production can align with halal and kosher certification requirements could pose a significant barrier to entry in many high-priority markets. In this regard, it is essential for religious bodies and third-party certification agencies to work closely with regulators and industry stakeholders to determine how cultivated meat and seafood cell line isolation and development may best align with their requirements.

TERMS AT A GLANCE

Primary cells: Cells harvested directly from a living tissue that can be grown as a culture outside the body under favourable conditions (*in vitro*) using a specialised medium containing essential nutrients and growth factors. Cultures may be adherent, where cells need to attach to a surface such as plastic or glass, or they may be in suspension, where cells are anchorage-independent and freely float in cell culture media.

Cell line: An established cell culture that is capable of proliferating across many generations *in vitro*. Over time, continuously passaged cell lines may acquire distinct genotypic and phenotypic characteristics, such that each cell line established from a single cell type and common tissue source may be unique in its metabolism and behaviour. This necessitates thorough characterisation of cell lines, particularly for the ability to proliferate and differentiate, while assessing their suitability for cultivated meat production.

Immortalised cell line: A cell line that has developed an ability to bypass senescence (an arrest in the ability of a cell to divide) and proliferate indefinitely under optimal culture conditions *in vitro*, either through spontaneously acquired or artificially engineered genetic variations.

Genetic engineering: The process of intentionally altering the DNA of, or genetically modifying, a cell or an organism to obtain desired functional or phenotypic properties.

Company Information and Current Business Focus

A majority of the companies saw cultivated meat as their core business currently ($n = 36$; 82%) or potentially in the future ($n = 5$; 11%), while only a few did not ($n = 3$; 7%).^[1] The main applications of cell lines or primary cells by these companies are indicated below (Fig 1), with many companies working across multiple functions. The most prominent are business-to-consumer (B2C) companies planning to develop and directly sell cultivated meat to consumers ($n = 28$; 64%). A considerable number are business-to-business (B2B) companies focusing on supplying B2C companies with various process inputs, such as media ingredients ($n = 12$; 27%), cell lines ($n = 10$; 23%), bioprocessing equipment ($n = 5$; 11%), and scaffolding technology ($n = 3$; 7%). A few companies ($n = 6$; 14%) aim to function as contract development and manufacturing organisations (CDMOs), while a smaller number ($n = 5$; 11%) provide ancillary services, such as artificial intelligence (AI)-assisted predictive modelling of cell behaviour and cell characterisation services, for cultivated meat production.

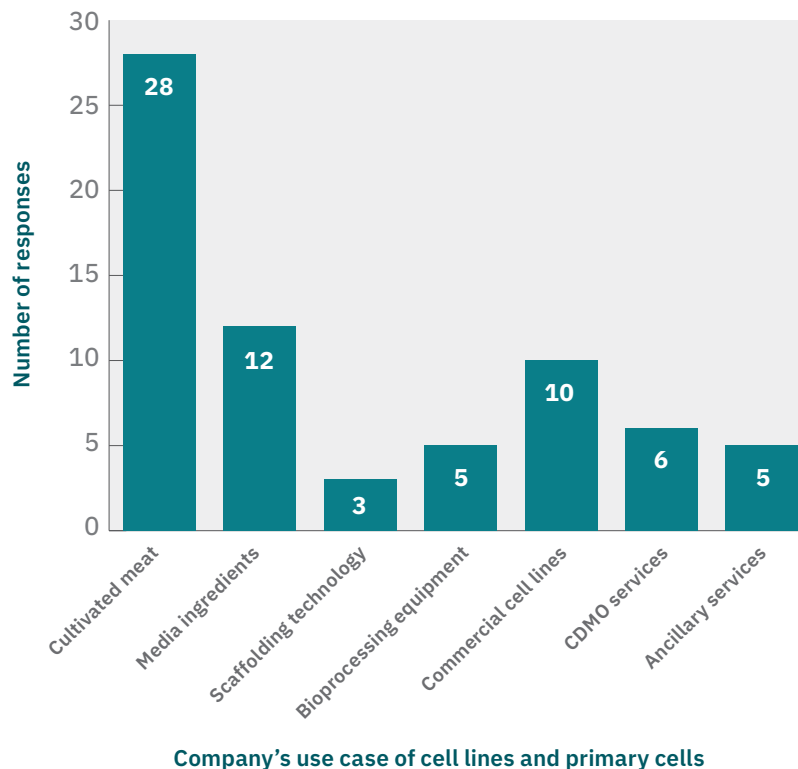


Fig 1: My company's use of cell lines or primary cells related to the cultivated meat sector is best described as _____ (select all that apply) (no. of respondents = 44)

Cell Line Sourcing and Usage

SECTION AT A GLANCE

- Most companies expressed a strong interest in acquiring cell lines from both terrestrial and aquatic species.
- Almost half of the companies currently rely solely on slaughtered animals for sourcing cells, with the desire to source more cell lines from live animals in the future.
- The use of a variety of cell types was reported, with myoblasts and fibroblasts being the most commonly used. For terrestrial species, this is likely driven—at least in part—by challenges in procuring and culturing embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Myoblast/myosatellite lines were found to be the most difficult to source for aquatic species compared to ESCs and iPSCs.
- Although almost half are open to purchasing off-the-shelf options, companies reported that they are largely using primary cells or cell lines harvested and developed in-house due to the unavailability or lack of access to suitable cell lines from repositories or government-sponsored cell banks. This represents a white space opportunity for B2B cell line providers to capitalise on and lower barriers to market entry for cultivated meat companies.

SPECIES OF INTEREST GENERALLY FOLLOW COMMERCIAL CONSUMPTION TRENDS

While some companies expressed an interest in acquiring cell lines from either terrestrial ($n = 7$; 16%) or aquatic ($n = 9$; 21%) species, most companies were interested in obtaining cell lines from both terrestrial and aquatic species ($n = 27$; 63%).^[2] Furthermore, most companies also specified that they work with cell lines from multiple species. For instance, 20 out of 34 companies (59%) that work with terrestrial species highlighted that they work with at least two species.^[3] Similarly, 20 out of 36 companies (56%) that work with aquatic species reported that they work with at least two species.^[4]

The highest-priority species from each category for cell line derivation are shown in the following charts. For terrestrial species (Fig 2), cows emerged as the top choice ($n = 21$; 70%). This aligns closely with data outlined in a recent **life cycle assessment** that showed conventional beef production has the most significant environmental implications compared to other terrestrial species, which may explain why bovine cell lines are a priority for cultivated meat companies. Pig ($n = 12$; 40%) and sheep/lamb ($n = 11$; 37%) were also deemed as high priority. Duck ($n = 2$; 7%), turkey ($n = 1$; 3%), and rabbit ($n = 1$; 3%) were of lowest priority. Although a few companies also selected “other avian species” ($n = 4$; 13%), details of the species of interest were not specified.

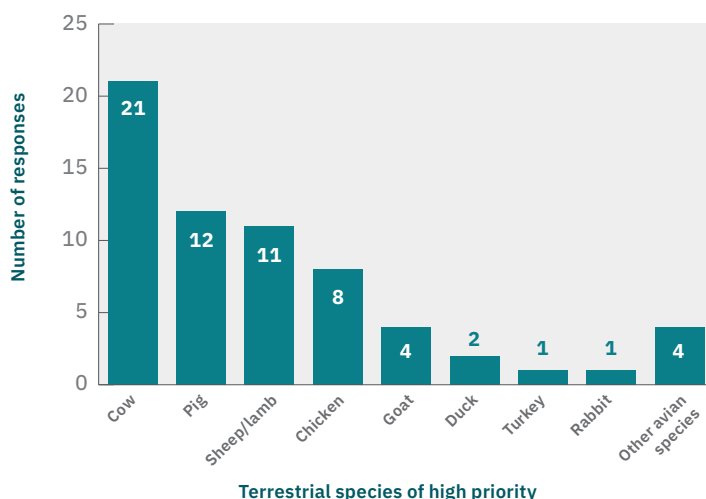


Fig 2: The category of terrestrial species that are the highest-priority and that my company has difficulty sourcing appropriate cells for is _____ (select up to four) (no. of respondents = 30)

For aquatic species (Fig 3), salmon ($n = 14$; 56%), tuna ($n = 13$; 52%), and shrimp ($n = 8$; 32%) emerged as high-priority species, followed by crab ($n = 6$; 24%) and lobster ($n = 5$; 20%). Companies also ranked finfish (other than salmon and tuna) ($n = 9$; 36%), crustaceans (other than shrimp, crab, and lobster) ($n = 5$; 20%), bivalve molluscs (such as oyster, clam, and scallop), and cephalopods (such as squid, octopus, and cuttlefish) ($n = 4$; 16%) as high priority.

Considering that a large number of companies deem many common terrestrial and aquatic species as high priority, there is almost certainly a significant duplication

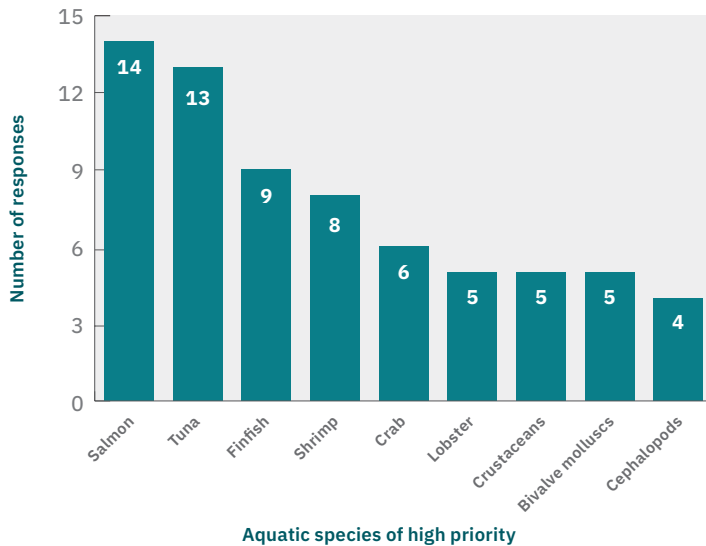


Fig 3: The category of aquatic species that are the highest-priority and that my company has difficulty sourcing appropriate cells for is _____ (select up to four) (no. of respondents = 25)

of effort with regard to cell line derivation, adaptation, and optimisation. A greater focus on collaborative or service provider-based creation of cell lines with commonly desired traits and characterisation data discussed in later sections would allow for more efficient use of R&D resources. Furthermore, this presents an opportunity for companies to diversify their revenue streams and licence cell lines developed in-house to other companies, especially if a potential customer is focusing on a different product type or geography.

THERE IS A HEAVY RELIANCE ON SLAUGHTERED ANIMALS AS CELL SOURCES

Only a small number of companies sample cells solely from live animals ($n = 11$; 27%) (Fig 4). The remaining companies sample cells either from both live and slaughtered animals ($n = 10$; 24%) or only from slaughtered sources ($n = 20$; 49%), with many wishing to incorporate more cell lines harvested from live animals ($n = 11$, 25%) or from both live and slaughtered animals ($n = 23$, 52%) in the future.^[5] This sourcing has potentially significant implications for regulatory approvals and product labeling (see Page 15).

A majority of companies ($n = 31$; 76%) also indicated their interest in acquiring cell samples from farmers, ranchers, or fishers local to their region,^[6] and a considerable number had already arranged contracts to do so ($n = 12$; 43%).^[7] However, we were unable to capture further insights into these contracts through the survey. Only one company disclosed an agreement that included

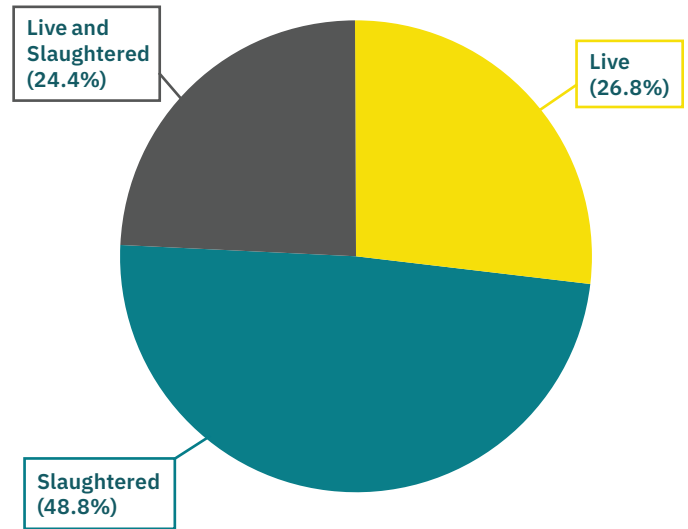


Fig 4: My company currently uses cell lines or primary cells sampled from _____ animals for commercial purposes (select one) (no. of respondents = 41)

cash payment and equity for the farmer, as well as cash payment for a veterinarian.^[8] A lack of publicly available information on the conditions outlined in such contracts presents a knowledge gap that may be preventing more companies from opting for them. Therefore, this presents an opportunity for the creation of an informative resource that outlines potential considerations and terms that could be adopted by interested parties. Enterprising livestock and seafood producers, perhaps in partnership with cell line providers, could also benefit from this resource, such that they are well-informed to enter competitive agreements that enable them to create a new revenue stream.

COMMONLY TARGETED CELL TYPES AND TISSUES ARE NOT SHIFTING

There is no apparent industry consensus on cell type selection as companies reported the use of a variety of cell types (Fig 5), with multiple often used in parallel. Over the next 12 months, companies are primarily aiming to produce cells constituting skeletal muscle, fat, and connective tissue, but in some cases are also producing cells for other tissues, such as bone, cartilage, vasculature, or cardiac muscle (Fig 6). Of note, cell lines and tissue types being employed roughly track with data collected in our 2021 [cell culture media and growth factor trends survey](#). The availability, commercial relevance, and/or ease of culturing these cell and tissue types have likely not shifted significantly since 2020 and corroborates our correspondence with industry and academia that obtaining appropriate cell lines remains a persistent challenge.

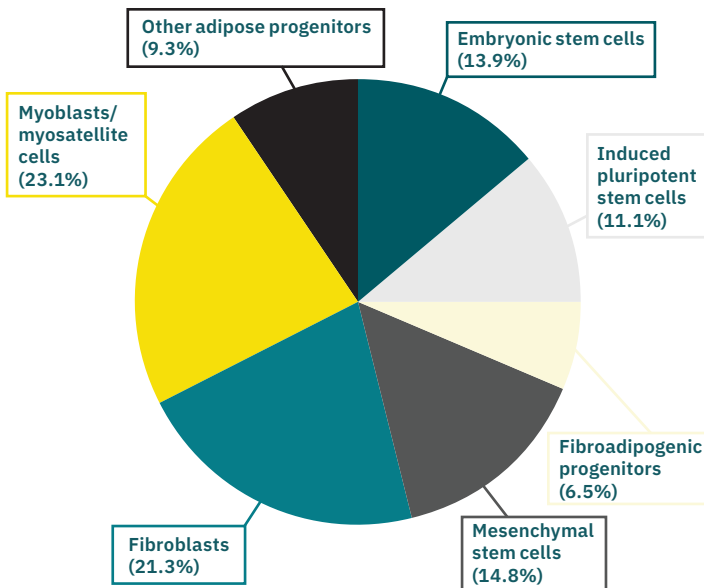
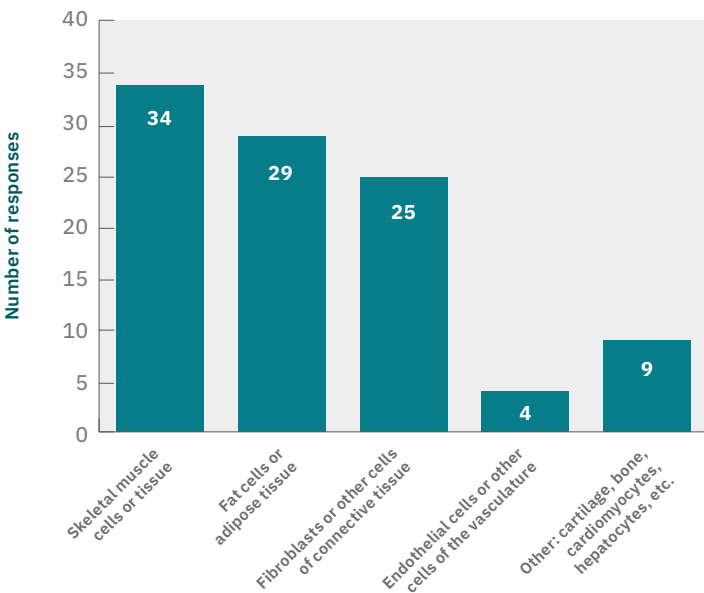


Fig 5: The principal cell type(s) my company currently works with is/are _____ (select up to four) (no. of respondents = 40)

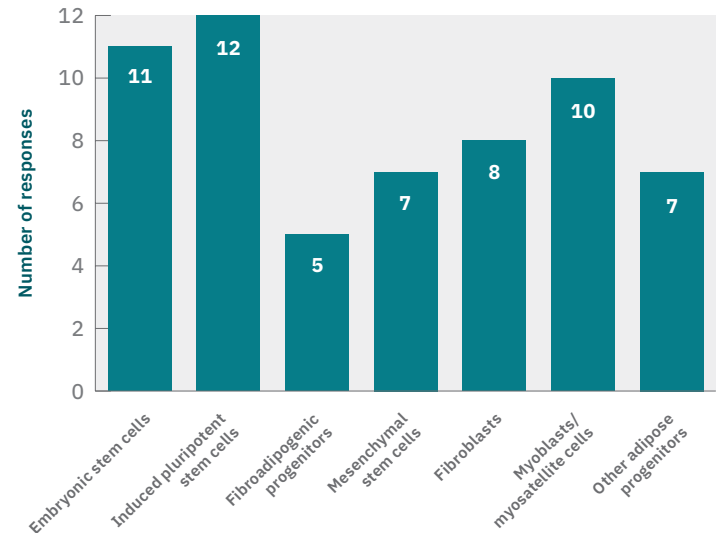


Cells projected to be produced by companies over the next 12 months

Fig 6: At my company, we produce or plan to produce _____ in the next 12 months (select all that apply) (no. of respondents = 40)

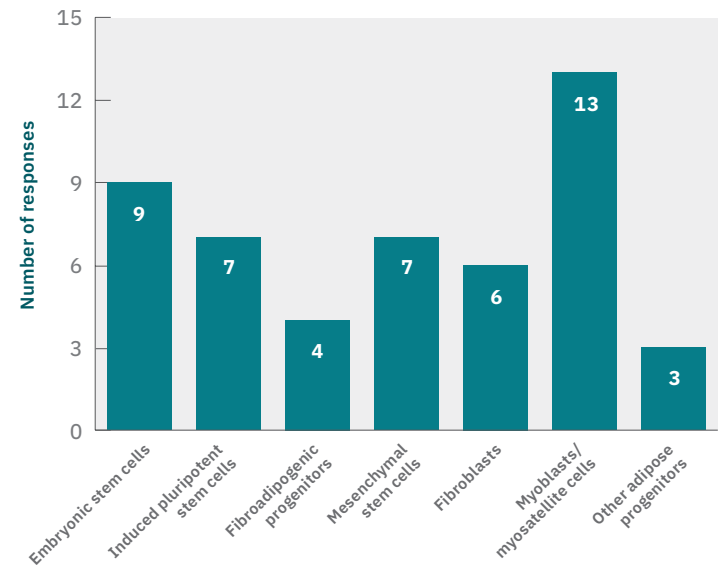
ESCs ($n = 11$; 44%) and iPSCs ($n = 12$; 48%) were the most prominent terrestrial cell types that companies are having difficulty procuring (Fig 7). This suggests that the current focus on using myoblasts ($n = 25$; 21%), fibroblasts ($n = 23$; 20%), and mesenchymal stem cells ($n = 16$; 14%) as seen in Fig 5 could be driven partially by the availability of source tissues, and relative ease of cell harvesting and culturing. By contrast, myoblasts/myosatellite cells were identified as the most difficult to source for seafood species ($n = 13$; 62%), followed by ESCs ($n = 9$; 43%) and iPSCs ($n = 7$; 33%) (Fig 8). This is

likely related to the inaccessibility of source animals, an absence of validated protocols for cell isolation and culture, a lack of established cell lines, as well as inadequate molecular tools, such as antibodies, for cell line characterisation.



Cell types from terrestrial species that are difficult to procure

Fig 7: The most desired cell type(s) my company has difficulty procuring or developing for terrestrial species is _____ (select up to four) (no. of respondents = 25)



Cell types from aquatic species that are difficult to procure

Fig 8: The most desired cell type(s) my company has difficulty procuring or developing for aquatic species is _____ (select up to four) (no. of respondents = 25)

SERVICE PROVIDERS FOR CELL LINE DEVELOPMENT AND BANKING ARE NEEDED TO INCREASE ACCESSIBILITY TO CELL LINES

Companies are largely using primary cells ($n = 33$; 77%) or cell lines ($n = 21$; 49%) that they have harvested and developed in-house.^[9] There is a noticeable absence of primary cells or cell lines being utilised from repositories or government cell banks. While companies largely wish to pursue in-house cell line development ($n = 34$; 79%), almost half are open to off-the-shelf options from repositories and government-sponsored cell banks ($n = 19$; 44%) or commercial providers ($n = 20$; 47%).^[10] By contrast, developing bespoke cell lines on contract, through non-disclosure agreements, for example, was the least appealing option ($n = 15$; 35%). A majority of the surveyed companies ($n = 36$; 82%) also specified a price range they are willing to consider for purchasing cells from repositories or commercial third parties (Fig 9). Close to 58% ($n = 21$) of the companies anticipate paying between \$250-1,000 (USD) per vial of cells (containing 1 million cells), with a smaller but considerable percentage considering \$1,000-3,000 ($n = 8$; 22%) or greater than \$3,000 per vial ($n = 7$; 19%) as fair market price. However, more data is needed to delineate how these price expectations may differ between cell lines intended for R&D use versus those licensed for commercialisation.

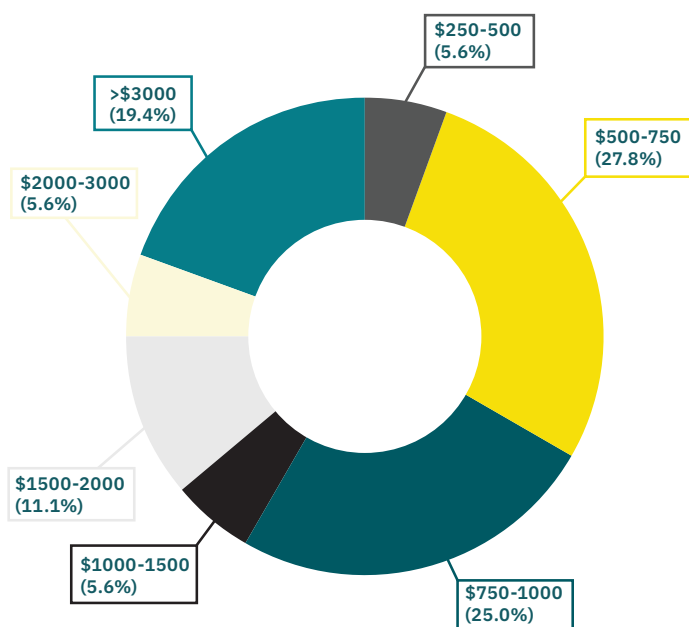


Fig 9: My company would anticipate paying approximately _____ (USD) for one vial containing 1 million cells, purchased from a repository or commercial third party (no. of respondents = 36)

Clearly, there is an unmet need where companies are open to off-the-shelf options and willing to pay premium prices for cells procured from repositories or government cell banks, but these are not currently available or do not have the right offerings to be utilised. A key example of how commercially available cell lines can bolster advancements in the industry comes from an industry leader in cultivated meat commercialisation—**GOOD Meat**. They used a cell bank-derived immortalised chicken fibroblast cell line (UMNSAH/DF1) that has been deposited in the American Type Culture Collection or ATCC since 1996.

In medical research, the creation of large-scale cell line biorepositories is often nationally sponsored, emphasising their fundamental importance in advancing research. The CentRe of Innovation for Sustainable banking and Production of Cultivated Meats (aka **CRISP Meats**) is a multi-institutional research programme hosted at the Agency for Science, Technology and Research (A*STAR), in collaboration with various local institutes of higher learning and research institutes. Funded by the Singapore Food Story Grant Call on Future Foods in 2021, this programme seeks to help address the cell line accessibility gap for cultivated meat production by exploring research collaborations with academic or corporate stakeholders interested in using cell lines deposited in their cell bank (see appendix for contact information). Government-backed projects, especially those that offer their cell lines for sale, are needed in other countries. Considering that geographic location is a barrier to accessing cell lines for companies (as discussed below), government-backed cell banks/repositories in strategically located countries would help overcome this. As the cultivated meat industry continues to mature and move away from full vertical integration as the standard business model, the emergence of more B2B players to service the cell line development space would allow companies to focus their efforts on different steps of the meat and seafood cultivation process. Furthermore, the inclusion of commercial cell lines developed by these B2B players in publicly available databases, such as GFI's **cell line database**, will play a significant role in increasing accessibility to cell lines across a wide range of species.

Three main barriers to accessing cell lines were highlighted by companies for both terrestrial and aquatic species: access to animals, proximity to slaughter and cell isolation facilities, and staff not being located in the right geography.^[11, 12] While this suggests that expertise and protocols are not a significant barrier for many companies, it is important to note that for less commonly

sought or cultured species, particularly for aquatic animals, relevant expertise and protocols are still hurdles. This is evidenced by the finding that isolation and culture protocols were a higher secondary barrier to accessing seafood cell lines ($n = 14$; 52%) compared to terrestrial ($n = 8$; 29%). Protocols and expertise may also become a larger issue as companies diversify from the most popular and well-characterised species, especially in the case of aquatic species. Key themes related to cell lines that emerged from a January 2023 **GFI expert workshop on fish cell cultures** included the difficulties posed by species differences, knowledge gaps related to fish cell biology, and the need for large-scale, collaborative efforts aimed at improving fish cell line availability. A related theme was that a lack of validated antibodies, annotated genome sequences, and other fairly basic research tools for fish species are key barriers for both academics and industry scientists.

In addition to easier cell line access, nearly 50% ($n = 21$) of the surveyed companies also stated that they were seeking suitable cell banking services for storage and internal company access or to be made available for commercial or non-commercial licensing purposes.^[13] This represents another commercial white space where B2B companies can offer services and infrastructure for cell banking, circumventing the need for cultivated meat companies to invest additional resources and talent towards this activity.

Establishing, Characterising, and Adapting Cell Lines

SECTION AT A GLANCE

- A minor proportion of companies currently uses genetically modified cell lines for R&D use and an even smaller proportion extend their use to commercial purposes. However, a majority of companies expressed a keen interest in using immortalised cell lines.
- Spontaneous immortalisation was deemed the preferred method to obtain immortalised cell lines suitable for both R&D and commercial use.
- Karyotyping, transcriptomic analysis, and whole genome sequencing were identified as the top three criteria that companies consider as minimum characterisation data needed after cell line immortalisation.
- The main features that need to be exhibited by a competitive cell line are immortalisation, a high proliferation rate and low doubling time, genetic stability, and growth in suspension.
- Successful cell line adaptation to growth in serum-free media was reported by a majority of the companies, with comparable or improved performance to serum-containing counterparts. In contrast, adaptation to growth in suspension remains a technical challenge and a time-consuming process with a low success rate.

LUKEWARM INTEREST IN EMPLOYING GENETICALLY ENGINEERED CELL LINES

Genetic engineering is the process of intentionally altering the DNA of, or genetically modifying, a cell to obtain desired functional and phenotypic properties. Within the context of developing cell lines for cultivated meat production, some of these properties include immortalisation, suspension growth, and low doubling time (see Page 12).

From a total of 39 responses, almost half of the surveyed companies stated that none of their work uses genetically engineered cell lines ($n = 19$; 49%). Of the remaining companies, 31% ($n = 12$) stated that the use of genetically engineered cell lines is restricted to R&D work alone, while a smaller proportion extended this to include commercial use ($n = 8$; 20%) (Fig 10). Response to the potential use of genetic modification technology for commercial purposes in the future was tepid, with most companies being unwilling ($n = 8$; 21%), unlikely ($n = 14$; 37%), or undecided ($n = 10$; 26%) about whether to produce products that would be classified as genetically modified by regulatory standards in their target markets.^[14] This is likely influenced by variable regulatory approval processes in different jurisdictions as well as variable consumer perceptions of genetically engineered food products.

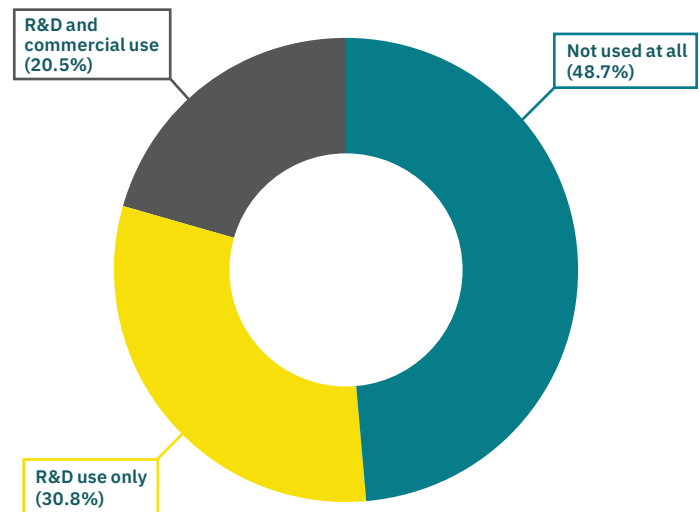


Fig 10: My company uses genetic engineering methods on our cell lines for _____ (no. of respondents = 39)

SPONTANEOUS IMMORTALISATION IS THE PREFERRED APPROACH TO IMMORTALISING CELL LINES

Although companies appear unwilling to use cell lines that may be termed as genetically engineered or modified for regulatory purposes, a majority of them expressed an interest in using immortalised cell lines. When asked what method of cell line immortalisation they would

prefer, 34 surveyed companies (77%) responded. Spontaneous immortalisation emerged as the method of choice to obtain immortalised cell lines for R&D ($n = 24$; 71%) or commercial ($n = 27$; 79%) purposes (Fig 11). This may be due to the fact that spontaneously immortalised cells are generally not considered genetically modified from a regulatory perspective and companies would still likely have access to **markets with conservative regulations** on genetic modification, such as the European Union (EU), Russia, India, and South American nations including Peru and Venezuela.

The second preferred option was the use of non-integrating expression approaches, such as via Sendai virus or synthetic messenger RNA (mRNA) ($n = 16$; 47%), for example, again likely due to its greater likelihood of avoiding potential regulatory issues with permanent genome alteration. Permanent genome alteration was the least preferred method for immortalising cell lines intended for R&D ($n = 13$; 38%) or commercial ($n = 10$; 29%) use. It is important to highlight that this survey did not explicitly poll for interest in gene editing—using CRISPR/Cas9, for example—as a separate method to generate immortalised lines. With countries such as the **U.S.** and **Japan** not necessarily requiring gene editing to undergo genetic modification approval processes for agriculture and food, and more countries such as the **United Kingdom** (UK) leaning in this direction, we may see a global increase over the coming years in the use of gene editing as a tool in the cultivated meat production process. This presents an opportunity for regulators across different regions to align regulatory guidelines related to cell lines developed via gene editing, to allow for streamlined assessment such as the implementation of a **shared genetically modified food safety assessment** by Health Canada and Food Standards Australia New Zealand (FSANZ).

A dissuasion to use methods other than spontaneous immortalisation may stem from a lack of available information regarding regulatory considerations needed to seek approval of cell lines immortalised using these approaches (see Page 16 for more details). In particular, an absence of consensus for characterisation criteria needed to assess performance and ensure the safety of immortalised cell lines may present a significant barrier to the adoption of non-spontaneous approaches, which may be more time- and resource-efficient depending on the species and cell type in consideration. Therefore, coordinated efforts are needed to establish industry standards and/or critical quality attributes for cell line characterisation, such that companies are empowered to employ a wider variety of experimental tools to generate

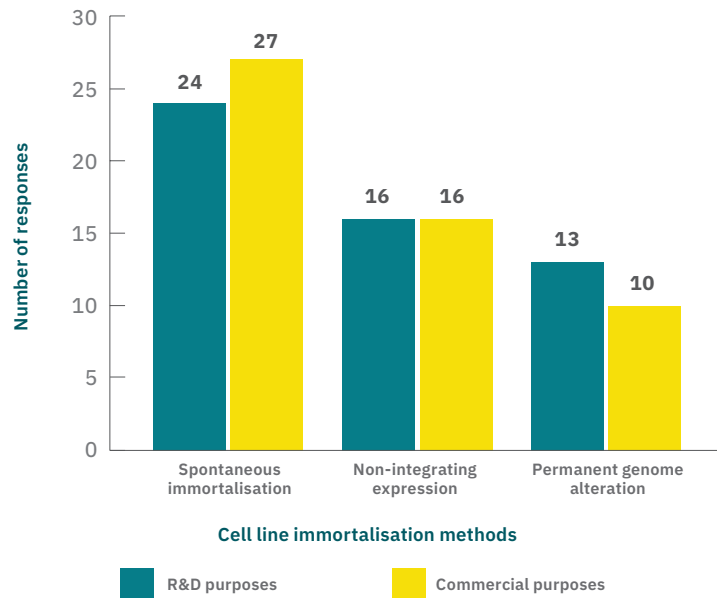


Fig 11: My company would use cell lines immortalised using the following methods (select all that apply) (no. of respondents = 34)

superior quality cell lines that are regulatory-compliant prior to formally seeking approval. This is an important consideration as the propensity to undergo spontaneous immortalisation **varies between species and cell type** and the associated genetic variation may alter the biology of the cells in unpredictable ways. Therefore, barriers to using other appropriate approaches need to be addressed to allow for more diverse and optimised cell line development as the sector matures.

COMPANIES DESIRE WELL-CHARACTERISED CELL LINES WITH SPECIFIC PROPERTIES

To better understand the characterisation criteria that companies employ for cell line development, they were asked to specify the characterisation criteria that they require data for as a minimum versus characterisation criteria that would ideally like to obtain data for, prior to using an immortalised cell line for commercial applications. Karyotyping of the immortalised cell line ($n = 24$; 71%), transcriptomic analysis after immortalisation ($n = 19$; 56%), and whole genome sequencing after immortalisation ($n = 18$; 53%) were identified as the top three minimum characterisation criteria required (Fig 12). However, responses were high for all characterisation criteria that companies would ideally like to have access to data from, with at least 20 companies (54%) opting for each approach provided (Fig 13).

To acquire insights into what properties companies are looking for in their cell lines, they were asked to specify

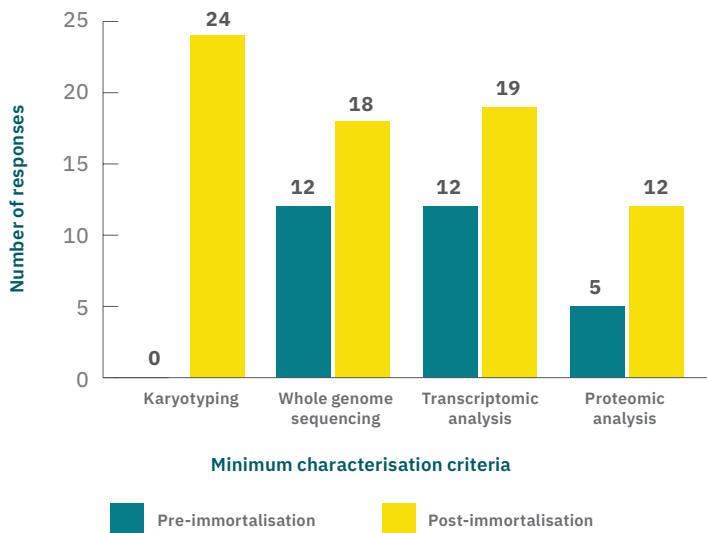


Fig 12: My company would require at a minimum _____ characterisation data for cell lines for commercial applications (select all that apply) (no. of respondents = 34)

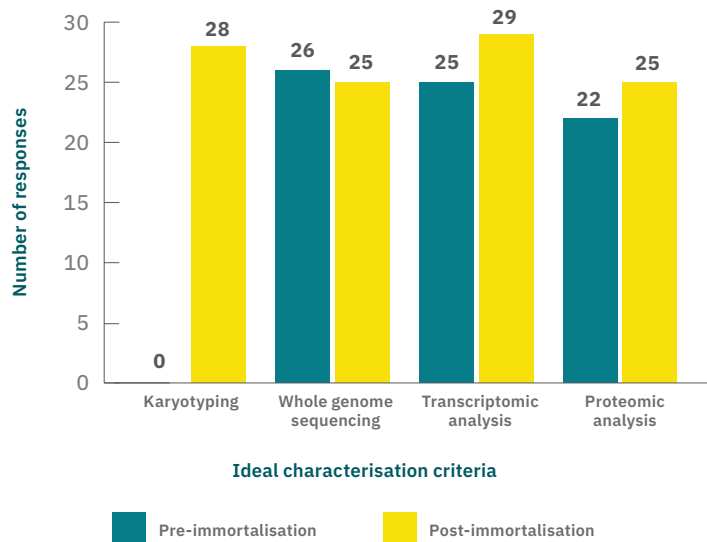


Fig 13: My company would ideally have access to ____ characterisation data for cell lines for commercial applications (select all that apply) (no. of respondents = 37)

characteristics they deemed as most important to be demonstrated by a new cell line. A high proliferation rate with a doubling time lesser than 24 hours ranked as the most important characteristic (Box 1). When asked to specify doubling times they believe are achievable,^[15] only three companies (8%) selected a doubling time greater than 28 hours as their target, and five companies (13%) selected 24-28 hours. In contrast, 15 companies (38%) specified 20-24 hours as their target range. Additionally, six companies (15%) specified 16-20 hours, seven companies (18%) specified 12-16 hours, and three companies (8%) specified 8-12 hours as achievable targets. Other desirable characteristics include genetic stability across multiple generations, immortalisation,

non-adherent growth in suspension, and strong differentiation potential towards target cell types. Most of these are unsurprising as key considerations, although the interest in non-adherent growth is noteworthy as an indicator that bioprocess considerations related to adherent cell growth may be a common concern in the industry. Furthermore, companies also outlined the minimum number of cumulative population doublings (CPDs) to be exhibited by a cell line for commercial production (Fig 14): 22 companies (58%) specified 21-60 CPDs, while a much smaller number opted for 61-80 CPDs ($n = 7$; 18%) or greater than 80 CPDs ($n = 9$; 24%) as a minimum.

Cell line characteristic	# of positive responses
High proliferation rate with a doubling time of < 24h	38 (93%)
Genetic stability across multiple generations	28 (68%)
Immortalisation	22 (54%)
Non-adherent growth in suspension culture	22 (54%)
Strong target cell type differentiation potential	20 (49%)
Metabolic efficiency	14 (34%)
Resilience to shear stress	6 (15%)
Resilience to, or limited accumulation of, toxic metabolic byproducts	5 (12%)

Box 1: My company would deem the most important commercial characteristics for new cell lines to demonstrate to be _____ (select up to four) (no. of respondents = 41)

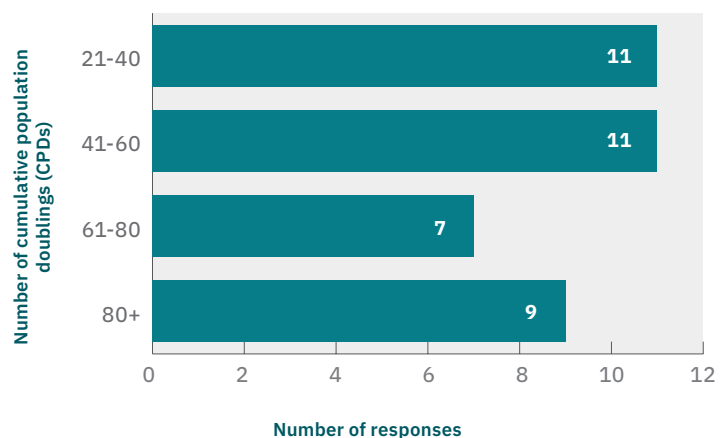


Fig 14: My company would require a cell line to exhibit a minimum of _____ population doublings without a reduction in proliferation rate or differentiation capacity (where relevant) for commercial production (no. of respondents = 38)

CELL LINE ADAPTATION IS A HURDLE FACED BY MANY COMPANIES

A majority of the surveyed companies confirmed successful adaptation of at least one cell line to grow in serum-free conditions ($n = 28$; 64%)^[16]—most in six months or less.^[17] Out of these companies, 56% reported comparable ($n = 15$) performance to counterpart cell lines grown in serum-containing conditions, and 18% ($n = 5$) reported improved performance (Fig 15). A small number of companies reported a loss in performance within a 10% range compared to serum-containing counterparts ($n = 5$; 18%), and a smaller proportion reported a loss in performance of greater than 10% upon adapting to serum-free conditions ($n = 2$; 7%). Companies open to exploring B2B opportunities to licence out their serum-free cell lines would be highly sought-after in the industry and are encouraged to enlist their cell lines in GFI’s [cell line database](#).

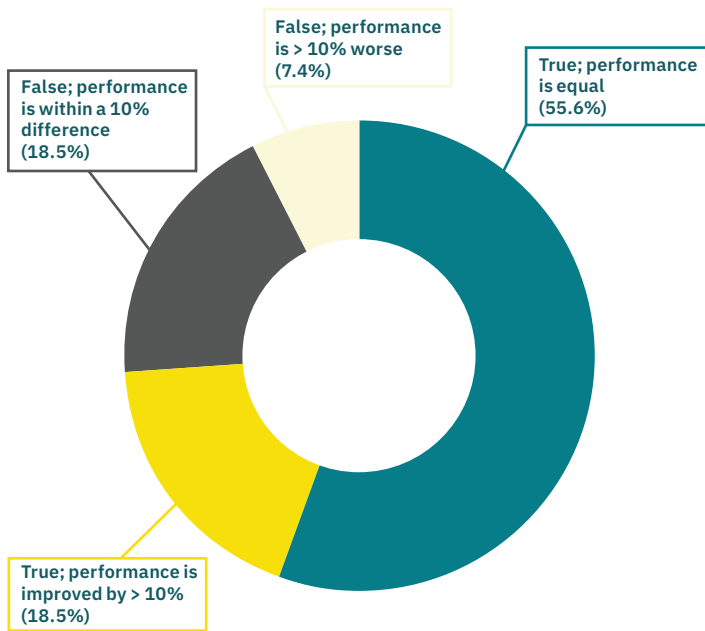


Fig 15: My company has achieved serum-free cell growth characteristics that are equal to or better than cell growth in serum-containing media (no. of respondents = 27)

Adaptation to growth in suspension remains a technical challenge, with more than half of the surveyed companies ($n = 22$, 54%) yet to report success.^[18] Of those that have been successful, a considerable number reported at least an equal ($n = 6$; 35%)—if not improved ($n = 8$; 47%)—growth of suspension cultures in comparison to adherent counterparts (Fig 16). This is important to highlight given that non-adherent growth in suspension culture ranked highly in the most important cell line characteristics (Box 1). Cell line providers or researchers who can tap into this niche and provide cell

line adaptation as a service would likely receive significant interest from the industry. Suspension cultures can offer advantages over adherent counterparts, especially when scaling up production, such as greater cell yield, optimised mass transfer, simplified harvesting of the cell mass, and not requiring a substrate or microcarriers for cell growth. Therefore, open-access research publications that share methodologies and protocols for successful adaptation of cell lines to growth in suspension, such as a recent [paper](#) published by Believer Meats in *Nature Food*, are highly beneficial for the industry and can expedite progress towards scaling up while preventing duplication of efforts.

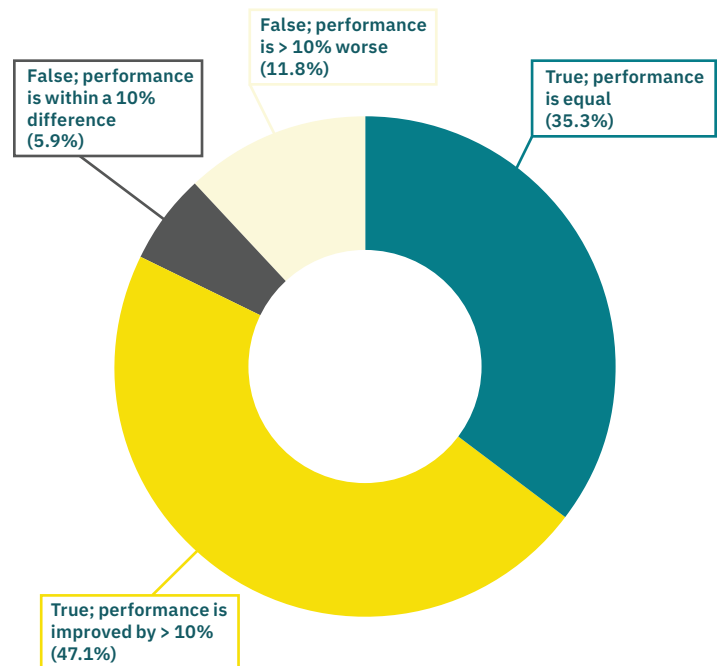


Fig 16: My company has achieved suspension cell growth characteristics that are equal to or better than previous growth in adherent culture (no. of respondents = 17)

This data can help inform B2B cell line providers of valuable documentation and services they could target to elevate the quality and appeal of their offerings. The availability of well-characterised, suspension-adapted, commercial cell lines with desirable functional and phenotypic properties circumvents or reduces the need for a cultivated meat company or academic research lab to invest additional resources for cell line development. It will also lower the barrier to entry for startups that would prefer not to develop cell lines in-house or do not have the relevant expertise, for example, if they are focused on culture media, scaffolding, or bioprocess innovation.

Regulatory Documentation and Testing

SECTION AT A GLANCE

- The U.S. and Singapore far exceeded all other markets that companies seek to understand or apply for regulatory approval in to sell their cultivated meat products.
- A majority of the companies indicated that their understanding of regulatory requirements for cell line approval in high-priority markets is either limited or lacking due to the unavailability of relevant information, particularly with regard to cell line derivation, characterisation, and safety testing requirements.
- There is a strong desire to generate cell lines that satisfy regulatory requirements under different religious and cultural contexts. Companies expressed a general preference for utilising cell lines that are compatible with both halal and kosher certifications, to widen their global market reach and potential consumer demographics.
- In obtaining the minimum background information required for seeking regulatory approval of cell lines, companies deem documentation related to the health status of source animals and specifics of the tissue sample or biopsy acquired as a high priority. Details regarding the environment or habitat where the source animal was raised were also considered significant.
- Documentation pertaining to allergenicity profiling and adventitious agent testing of cell lines was also considered a requirement by a majority of the companies.

THE U.S. AND SINGAPORE WERE DEEMED HIGH-PRIORITY MARKETS FOR A MAJORITY OF COMPANIES

To better understand regulatory regions of interest, companies were asked to specify up to three of their highest-priority markets (Fig 17) in which they are considering or actively seeking regulatory approval to sell their products. The U.S. appeared at the top, with 67% ($n = 29$) deeming it a market of high priority, followed closely by Singapore at 63% ($n = 27$). A much smaller proportion of companies indicated interest in other markets, such as the E.U. ($n = 11$; 26%), China ($n = 10$; 23%), and the UK ($n = 9$; 21%). Apart from the regions visible in the figure to the right, a handful of responses ($n = 7$; 16%) specified “other” regions of interest, such as Latin America, Southeast Asia, Africa, and the United Arab Emirates.

The significant difference in the proportion of companies opting for markets other than the U.S. and Singapore may be attributed largely to a fundamental gap in understanding and awareness of region-specific regulatory requirements as well as the proactive nature of both the U.S. and Singapore in establishing their relevant regulatory regimes. Out of 35 responses, only 10 companies (29%) expressed confidence in their understanding of

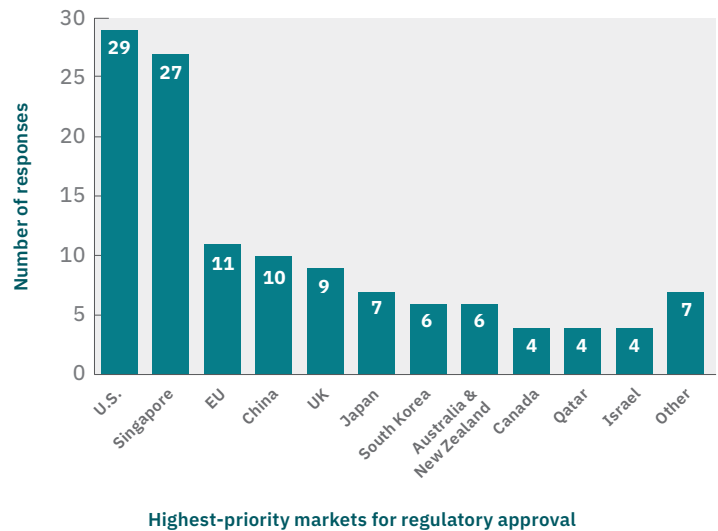


Fig 17: My company’s highest-priority markets in which we are actively planning to seek regulatory approval to sell cultivated meat are _____ (select up to three) (no. of respondents = 43)

documentation and testing requirements for obtaining regulatory approval in their high-priority markets.^[19] The remaining companies stated that their knowledge was lacking ($n = 14$; 40%) or limited ($n = 9$; 26%) due to the unavailability and inaccessibility of relevant information, or because they were yet to investigate regulatory requirements ($n = 2$; 6%).

GFI maintains a **list of technical consultants** that includes some regulatory consultants, though a clear need exists for more experts with appropriate technical and regulatory knowledge to assist companies in navigating approvals in different markets. Increased efforts towards educating regulators to bridge existing knowledge gaps on cultivated meat technology and relevant production processes will aid in developing robust regulatory guidelines. This will become increasingly relevant as more markets set up regulatory regimes, as highlighted by the recent U.S. Food & Drug Administration (FDA) “no questions” letters for **UPSIDE Foods** and **GOOD Meat**, and recent developments in **Japan, South Korea, Singapore**, and **Australia and New Zealand**.

COMPANIES FACE A PERSISTENT LACK OF CLARITY ON REGULATORY REQUIREMENTS AND CERTIFICATION CONSIDERATIONS FOR CELL LINES

To gain deeper insights into the inadequacy of regulatory information facing industry players—particularly for approving cell lines—companies were asked to highlight their key knowledge gaps.^[20] Information regarding the assessment of techniques used to establish cell lines, such as immortalisation or iPSC reprogramming, as well as subsequent testing and characterisation requirements of cell lines, were identified to be the main knowledge gaps by more than half of the companies ($n = 37$; 65%). Lack of clarity on documentation requirements related to animal/tissue sourcing, and isolation and cell line storage, were also highlighted as barriers to seeking regulatory approval. It is important to note that this survey was conducted prior to the “no questions” letters issued to UPSIDE Foods and GOOD Meat by the FDA, when regulatory considerations were less well understood. However, given that two-thirds of the companies highlighted Singapore as a target market (Fig 17)—which first approved a product in 2020—it is concerning that lack of clarity on requirements remains an issue. This demonstrates that regulators must continually optimise their public and private communication strategies with cultivated meat companies and other regulatory bodies to provide clear and aligned guidance. Continued multi-lateral work such as the UN/FAO **Food Safety Aspects of Cell-Based Food** publication launched in April 2023 will help ensure regulatory requirements align with the realistic hazards associated with cultivated meat production.

A majority of surveyed companies expressed a preference to generate cell lines that adhere to religious and cultural dietary laws and can be deemed halal or kosher

(Fig 18-19). However, the absence of explanatory guidelines on obtaining these certifications poses a significant barrier to entry in many high-priority markets. Out of 39 responses, only a minority of companies confidently understood what requirements would exist for halal ($n = 7$; 18%) and kosher ($n = 5$; 13%) certification, with a large number unable to access information or yet to attempt to understand the requirements for either.^[21, 22] As of January 2023, Aleph Farms is the only company that has **received confirmation** that their products may be deemed kosher, which highlights the need for increased dialogue between cultivated meat companies and religious authorities. The current discussion around religious labelling of cultivated meat suggests that some Islamic religious authorities (noting that interpretations are made by each nation’s relevant organisation(s)) may not approve halal certification for cells taken from an animal prior to slaughter through appropriate methods. This could become a major issue given that 77% of surveyed companies’ future cell line development plans include incorporating cells obtained from live animals or from both live and slaughtered animals.^[5] While this may not be an immediate driver for many companies focusing on the U.S., Singapore, Japan, or European nations, halal certification is important to a large segment of the global population, particularly in South and Southeast Asia. In this regard, it is essential for religious bodies to work closely with regulators and industry stakeholders to determine how technologies and processes integral to cultivated meat production may align best with religious requirements across multiple regions.

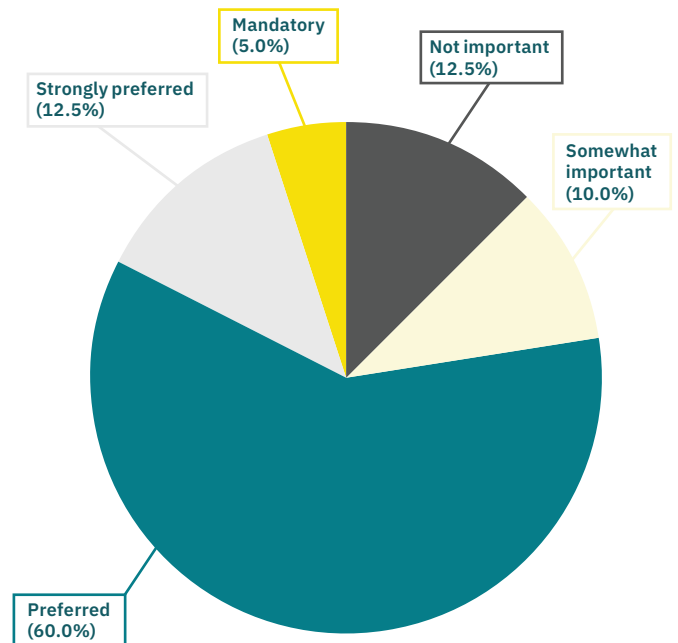


Fig 18: My company would rank cell lines that could comply with halal certification for relevant species as _____ (no. of respondents = 40)

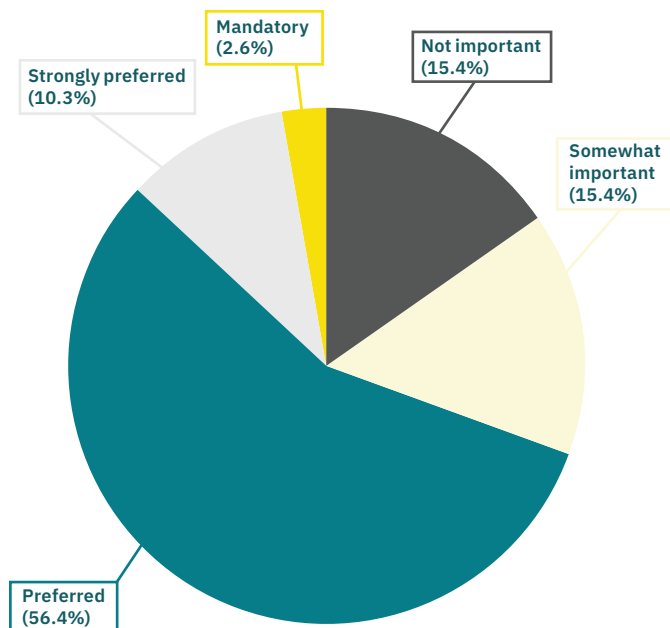


Fig 19: My company would rank cell lines that could comply with kosher certification for relevant species as _____ (no. of respondents = 39)

DONOR ANIMAL PARTICULARS, HEALTH STATUS, AND FARM DETAILS ARE ESSENTIAL FOR COMPANIES WHEN ACQUIRING SAMPLES

Companies were asked to specify the minimum background information they require when acquiring a cell

line or a tissue sample, for the purpose of traceability and in accordance with preparing dossiers for regulatory submission (Box 2; column 1). From the criteria provided, individual animal details were the top priority for a majority of companies. Out of 40 responses, 39 companies (98%) required the individual details of the donor animal, such as age, sex, weight, disease status, health, and vaccination history. Close to half ($n = 19$; 48%) extended their requirements to include veterinarian inspection of the animal pre- and post-mortem.

Tissue sample or biopsy details, such as source tissue type and anatomical location, also ranked as a high priority for 33 companies (83%). However, documentation detailing whether pain relief or euthanasia was administered to the animal at the time of sample acquisition was of low priority ($n = 12$; 30%). Furthermore, one company detailed (as free text) that they needed to document whether the animal was alive or slaughtered at the time of sample collection, which as mentioned previously may be a key consideration for halal status of the subsequent food product.

Information regarding the source animal's environment and habitat was of moderate priority. Over half of the companies ($n = 24$; 60%) specified the need for details regarding the farm's geographical location, region, and farming system utilised. A lesser number ($n = 18$; 45%) were interested in documenting whether there was a

Background information criteria	# of positive responses (column 1)	# of positive responses (column 2)
Individual details of the animal (age, sex, weight, disease status, health and vaccination history)	39 (98%)	40 (100%)
Tissue sample or biopsy details (including source tissue type and anatomical location)	33 (83%)	34 (85%)
Geographical location of the farm and farming system in use	24 (60%)	31 (78%)
Veterinarian inspection of the animal pre- and post-mortem	19 (48%)	27 (68%)
History of disease transmission within the source herd	18 (45%)	26 (65%)
Confirmation of whether the animal was raised in a pathogen-free environment	18 (45%)	27 (68%)
Documentation of pain relief or euthanasia administered to the animal	12 (30%)	27 (68%)

Box 2:
 Column 1: When acquiring a cell line or sample, my company would require at a minimum _____ as background information (select all that apply) (no. of respondents = 40)
 Column 2: When acquiring a cell line or sample, my company would ideally have access to _____ as background information (select all that apply) (no. of respondents = 40)

history of disease transmission within the source herd or validation that the animal was raised in a pathogen-free environment.

The priorities represented here are indicative of the types of information that companies have easy access to and are able to obtain depending on their capabilities, as well as general documentation practices followed by the farmer/breeder that samples are sourced from. When asked to specify all the background information that they would ideally want to obtain (Box 2; column 2), at least 26 companies (65%) opted for access to each of the options provided.

ADVENTITIOUS AGENT TESTING AND ALLERGENICITY PROFILING ARE IMPORTANT FACETS OF CELL LINE CHARACTERISATION

To gain insights into how companies monitor the generation of pathogen-free cell lines, they were requested to specify the adventitious agent testing documentation they deemed necessary (Fig 20). A majority required testing documentation for mycoplasma ($n = 37$; 93%) and other bacteria ($n = 25$; 63%), as well as viruses ($n = 30$; 75%), particularly endogenous retroviruses ($n = 26$; 65%). Half of the companies ($n = 20$; 50%) also flagged testing for prions as a requirement. Beyond these, a significant number of companies highlighted the need to test their cell lines for other adventitious agents, if any, known to exist in the individual animal from whom cells were harvested ($n = 22$; 55%) or the species of origin ($n = 31$; 78%).

Companies were also asked to indicate their desire to obtain allergen profiling documentation for their cell lines.^[23] Only three of 39 companies (8%) did not consider allergen profiling as important. Most companies ($n = 26$; 67%) exhibited a preference for allergen profiling of their cell lines and a further 10 companies (26%) deemed it mandatory. Moreover, close to 92% of companies ($n = 34$) specified a desire for their cell lines to have the same, or reduced, allergenicity risk profile as compared to conventional animal counterparts.^[24] This feature may be an additional consideration to take into account for B2B cell line providers, to ensure that their products have desirable allergenicity profiles. It also highlights an opportunity for cultivated meat companies to partner with immunologists, clinicians, or other food and nutrition companies to better understand and characterise the allergenic properties of their cell lines.

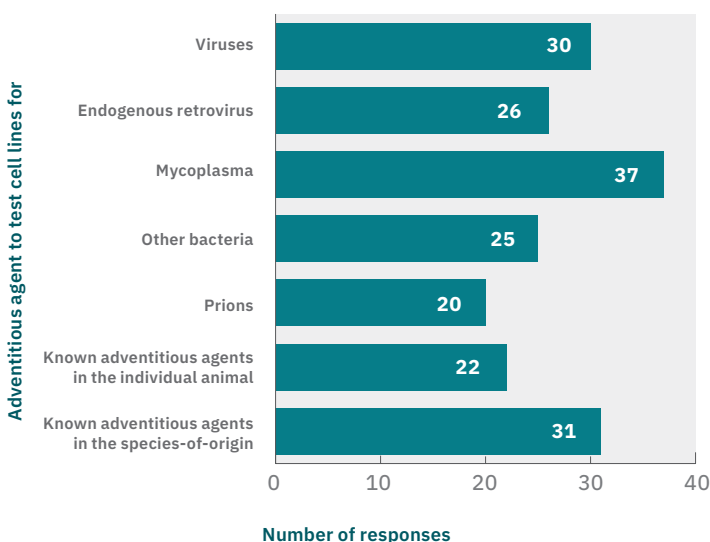


Fig 20: My company would require cell lines to have the following adventitious agent testing documentation (select all that apply) (no. of respondents = 40)

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Appendix

[1] My company views cultivated meat as our core business (no. of respondents = 44)

[2] My company is interested in obtaining cells from _____ (select all that apply) (no. of respondents = 43)

[3] The category of terrestrial species that are the highest-priority and that my company has difficulty sourcing appropriate cells for is _____ (select up to four) (no. of respondents = 30)

[4] The category of aquatic species that are the highest-priority and that my company has difficulty sourcing appropriate cells for is _____ (select up to four) (no. of respondents = 25)

[5] My company plans to obtain future cell lines or primary cells sampled from _____ animals for commercial purposes (no. of respondents = 40)

[6] My company acquires or plans to acquire cell samples from farmers, ranchers, or fishers local to our region (no. of respondents = 41)

[7] My company has arranged contracts with these individuals, including payments and/or future royalties on sales (no. of respondents = 28)

[8] Please share any specific details (free text) (no. of respondents = 1)

[9] My company currently uses _____ for R&D and/or product development (select all that apply) (no. of respondents = 43)

[10] My company is primarily considering obtaining future cell lines _____ (select all that apply) (no. of respondents = 43)

[11] The main barriers my company has to accessing cells from terrestrial species are _____ (select all that apply) (no. of respondents = 28)

[12] The main barriers my company has to accessing cells from aquatic species are _____ (select all that apply) (no. of respondents = 27)

[13] My company is seeking/using cell banking services including _____ (select all that apply) (no. of respondents = 42)

[14] My company would use cell lines for commercial purposes that are likely to be classified as genetically modified (based on your understanding of the regulatory jurisdiction(s) you plan to sell products and in which GM foods can be approved) (scale from 1 (never) to 5 (very willing)) (no. of respondents = 38)

[15] The cell doubling time my company is targeting and believes to be achievable for a cell line entering commercial production is _____ (no. of respondents = 39)

[16] My company has derived at least one cell line that has been adapted to growth in serum-free conditions (no. of respondents = 44)

[17] My company's average length of time required to generate a cell line that performs well in fully serum-free conditions is approximately _____ (no. of respondents = 24)

[18] My company has derived at least one cell line that has been adapted to growth in suspension/adherence independent culture (no. of respondents = 41)

[19] My company's understanding of the regulatory requirements for cell lines in our highest-priority market is _____ (no. of respondents = 35)

[20] My company's key knowledge gap regarding how the regulator in our highest-priority market will assess cell lines is _____ (select all that apply) (no. of respondents = 24)

[21] My company's understanding of the requirements for cell lines being certified as halal is _____ (no. of respondents = 39)

[22] My company's understanding of the requirements for cell lines being certified as kosher is _____ (no. of respondents = 39)

[23] My company would rank our desire for cell lines to have allergen profiling documentation as _____ (scale from 1 (not important) to 5 (mandatory)) (no. of respondents = 39)

[24] If allergen profiling is conducted, my company would require the cell line to have the same, or reduced, allergenicity risk profile as compared to its relevant animal counterpart (scale from 1 (not important) to 5 (mandatory)) (no. of respondents = 37)

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