Workshop 5: Advances in cell engineering and alternate expression systems

Attendees were broken up by table to discuss the advances, benefits and challenges of a variety of topics in this space. The summary of each table is as follows:

Table 1: Mammalian alternatives to CHO
Discussion focused on what were the pros and cons of alternative hosts and what would be the driving force to change hosts. Some of the considerations were a desire to move from ~10 g/L of product to ~100-200 g/L in order to reduce capital costs, bring down cost of goods and make biologics available in low- and middle-income countries. Other drivers were difficult to express proteins that might express better in alternative hosts. The possibility of identifying hosts that might secrete reduced amounts of host cell proteins was also seen as an attractive feature. However, it was perceived that there were not currently any better hosts out there. The regulatory advantage of CHO is significant as well as the large body of established technology and platforms processes focused on CHO. In addition, now that we have genetic and computational tools (sequenced genome, genome scale models, proteomic data, etc.) that seems a waste to give up. The general consensus was that an alternative would need compelling advantages to change.

Table 2: Microbial alternative hosts
The discussion focused on the pros and cons of utilizing a microbial host organism to express proteins that are more commonly produced in CHO or HEK. The summary of the benefits for these organisms included: potential lower cost of goods, less host cell protein and viral contamination concerns, shorter growth cycles enabling faster processes, ease of engineering and ability to leverage other cheaper carbon sources. On the contrary some of the challenges included: the non-mammalian glycosylation, generally lower productivity, the immunogenicity of the HCPs, economic barriers to implementation, and general industry reluctance to shift to a new expression system. Through the discussion several interesting possibilities emerged as potential uses or applications of these organisms. Transgenic plants or protists/algae could be considered for sustainability as they leverage natural sources of energy. Inducible expression systems could be leveraged to modify glycosylation in a way that would not be toxic to the cells. The ability to engineer these cells could result in generation of a 'minimal' cell that was built specifically for protein production or as a system for enhanced plasmid production. Overall, there is clearly a space for these organisms but it is unclear in the current state of the biologics industry.

Table 3: Alternative cell lines for cell and gene therapy products
The discussion overviewed the main cell host and expression systems used to produce gene therapy products (focusing on lentiviral and adeno-associated viral vector based products. HEK 293 cell lines and their derivatives is the current most use host system to generate AAV and LV vectors. Other cell lines as HeLa, 911, A549, BHK, EB6, Sf9, PerC6 and CAPT cells have been also used. The question if CHO could be used as a platform was also posed. It is known that CHO have low susceptibility for most of the viruses supposedly possessing high amount of restriction factors, further information and studies are required. After discussion HEK 293 cells were considered the most reliable and the cells where most accumulated knowledge exist in terms of process manufacturing for viral vector production up to date.
Two major expression systems are used for viral vector production, transient transfection and stable packaging or producer cells lines. Both systems will still continue to exist although one will be preferred over the other at specific stages of product development and production. Stable cell lines, in particular helper virus free are the ideal system when going to larger scales and when products are approved and transition to the market. Challenges on the current production of viral vectors were identified: the product titer yields and quality (namely on full/empty particles ratios), scalability of the process and stability of the producer cell lines. Strategies to improve the current manufacturing process may be use to further boost production as cell engineering (e.g. KO of antiviral responses, metabolic engineering, apoptosis) or the use of supplements (e.g. antiviral molecules, other additives).
The ideal cell line for AAV and LV production was also profiled. It should be a genetically stable cell line, able to grow in suspension serum-free cultures, able to grow at high cell densities, providing high titer yield and modular (e.g. enabling switch of expression cassettes for serotype swap).

Table 4: Cell engineering approaches to altering primary metabolism
This table focused on a few specific questions in the space to understand the challenges associated with modifying primary metabolism using genetic engineering. To understand the long term stability of the phenotype in culture it was recommended to test in a perfusion process to observe differences in growth, titer and productivity over extended number of generations that would align with your manufacturing process. Omics could be used to identify targets and markers that would help track these phenotypic changes on the genetic or metabolic level. The discussion also focused on how to get both optimal growth and productivity from these cell engineering approaches. Identifying factors that inhibit growth and engineering them out of the cell in combination with qp enhancers would be one way to achieve both. Mitochondrial content could also be increased through engineering to increase energy metabolism that would support the energy needed for higher growth and qp. Lastly, the discussion focused on when is the appropriate opportunity to knock out genes. If you are targeting a generic property of the cell it makes sense to engineer the host versus if it is a molecule specific target which should be tackled during cell line development. IT would be important to characterize the knock-out or knock-in for the IND and have a clear value proposition to get endorsement for this sort of modification which is probably more acceptable for product quality attributes like HCPs, glycosylation, etc.

Table 5: Directed evolution for host cell optimization
This group focused on assessing the interface between successful recovery (genotype) and desired function (phenotype) to map the outcomes of performing directed evolution experiments. To achieve the desired functionality one would start with a cell population that has a high degree of diversity and apply a high stress environment that forces the cells to evolve. To ensure successful recovery one could leverage custom expression vectors and implementation of anti-apoptotic genes like BAX/BAK followed by genome wide screens to measure genetic changes. Ultimately the ideal state would achieve the desired function and successful recovery that yield cells that are tolerant of shear stress, grow fast, hit high peak cell density and productivity, show apoptotic resistance, enhanced metabolism and be compatible with intensive process formats.

Table 6: Prime editing approach and related technologies
Prime and base editing can be used to generate knockouts with low indel and off-target effects (when comparing to genome editing). Can be thus used to study causal elucidation of cell line variations. Can be used as an engineering tool to delete or insert binding site motifs for expression, perform protein engineering in situ, or alter epigenetic sites/motifs. Additionally BE and PE can be used to generate libraries that can be used in 'directed evolution' approaches.

Table 7: Site specific integration
Site-specific integration (SSI) is used in cell engineered to develop stable cell lines aiming to find high expressing and stable chromosomal hot spots. Finding a hot spot is however hard. After identifying a good SSI this methodology presents several advantages as reducing clone screening, providing pool comparability and stable and predictable cell behavior. It was question if cell pools, as the ones established through SSI, could be used for BLA.
Predictability is the key with SSI technology. The idea is that homogenous SSI pools would behave comparably to SSI-derived clones (in terms of stability/titer/quality), thus supporting the use of pools throughout the regulatory process, from Phase I to commercial. The push for this kind of change needs to be a collective data-driven effort from industry and the scientific community to persuade regulatory agencies that the focus should be on product quality and safety, not whether the cell line was clonal or not. After all, CHO clones are widely disputed as being true clones since they have highly plastic genomes that undergo genetic drift over time.
Aside from cell line development, the SSI platform can also be used for R&D/discovery projects to, for example, assess the impact of gene expression or vector components on titer and key product quality attributes, because the variability from unknown/random integration sites is eliminated.

Table 8: Genome editing technologies (CRISPR/ZFN/TALENs)
There was significant interest in and use of gene editing technologies; however, the IP concerns around CRISPR made people reluctant to use it in any commercial product. MAD7 was discussed as an alternative to CRISPR.

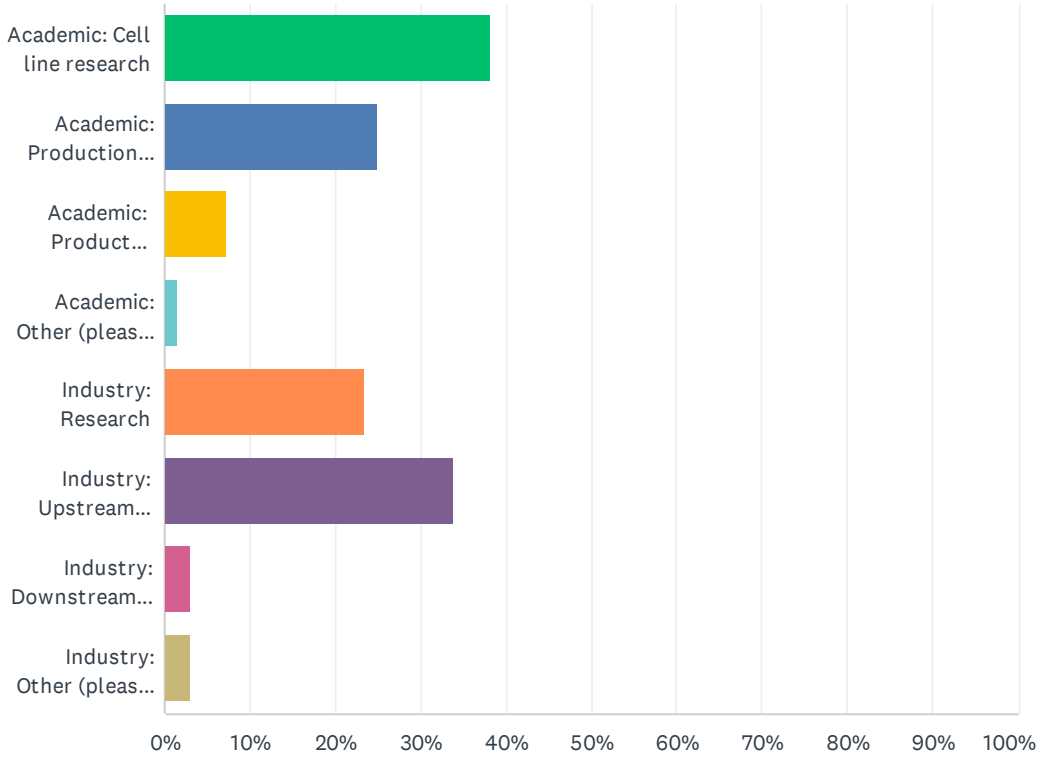
- We had a mix of both industry and academia at our table, and everyone has similar views of CRISPR, being that: it's great, and we all use it as quick proof of concept work, but no one will touch it when it comes to generating anything that could yield a commercial product.
- ZFNs/TALENs are suitable however they more intensive in their design and less flexible. Not as plug and play as a gRNA design
- MAD7 was the universal tool of choice amongst the 8-10 at our table (aside from myself, being from MilliporeSigma and having free access to ZFNs)
- This of course was due to Inscripta's very flexible and broad licensing structure associated with it
- Collectively were unsure of the TALEN IP, but the ZFN patent portfolio will largely expire from Sangamo in the next 1-3 years, and in the near term future could present a completely license/IP free solution for people, despite the less flexible targeting/design

Table 9: Genome editing to reduce host cell proteins
Approaches addressed include identifying specific proteins to knockout, particularly based on regulatory issues as well as the concept of creating a minimal CHO genome.

Table 10: Transposases for genome editing
A number of different transposases were discussed including PiggyBac, ATUM LPN, Sleeping Beauty, Directed Luck (Probiogen), Goldilock (Celltheon) and TNT. A number of issues were discussed including pools vs. clones, product quality attributes, stability, costs and freedom to operate and whether DOE was needed for optimization.
Janssen uses Leap-In and has a good term. Janssen did DOE with different DNA combination and raised average ~ 1g/L titer from maybe 5-7 g/L. Both Janssen and Eli Lilly (PiggyBac) saw increased titers at pool and clonal stages, also more stable clones evolved from transposase pools. No significant changes were observed for product quality attributes, so this is less of a concern for transposase usage. All transposases seem to work, so costs and freedom to operate is the main concern when people determine which system to use. Directed Luck (Probiogen) seems to give very low copies (1-2), so it could be a way to screen/engineer hotspot.

Q26 What do you do (select all that apply)?

Answered: 68 Skipped: 261



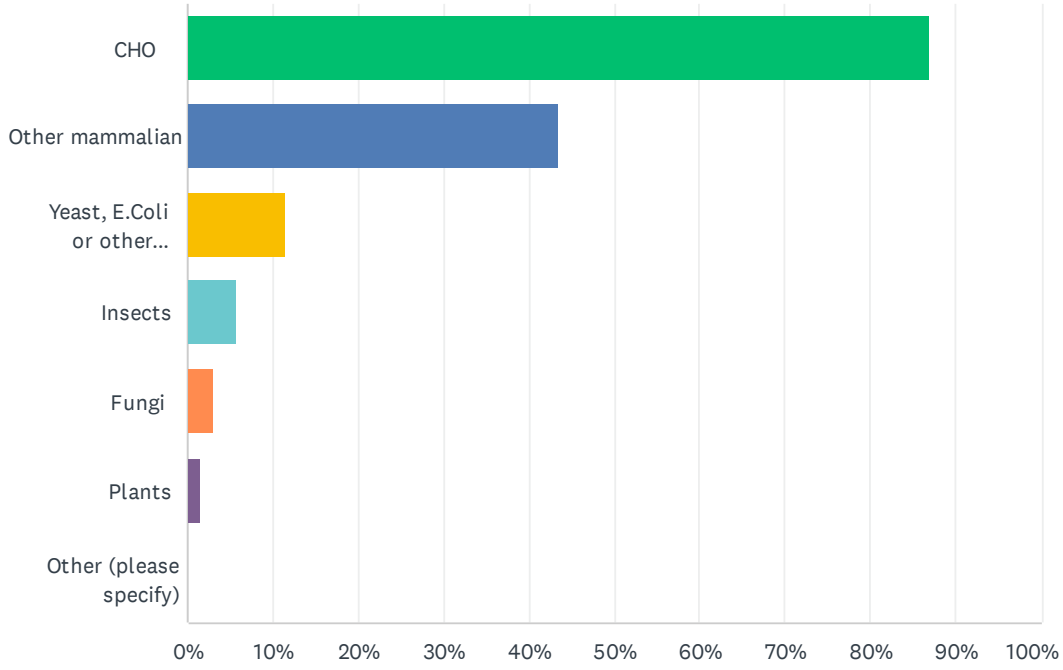
ANSWER CHOICES	RESPONSES	
Academic: Cell line research	38.24%	26
Academic: Production research	25.00%	17
Academic: Product research (e.g. specific drug candidate)	7.35%	5
Academic: Other (please specify below)	1.47%	1
Industry: Research	23.53%	16
Industry: Upstream production	33.82%	23
Industry: Downstream production	2.94%	2
Industry: Other (please specify below)	2.94%	2
Total Respondents: 68		

#	OTHER (PLEASE SPECIFY)	DATE
1	Bioinformatics	4/13/2023 4:38 PM
2	Field Scientist providing solutions	4/13/2023 3:46 PM
3	Cell line development	4/4/2023 2:09 PM
4	Industry Cell lIne Development	4/3/2023 7:42 PM

5	Up and downstream	4/1/2023 10:24 AM
6	Engineered cells for vastly higher protein or AAV productivity	3/31/2023 5:01 PM

Q27 Which host organisms do you work with (select all that apply)?

Answered: 69 Skipped: 260

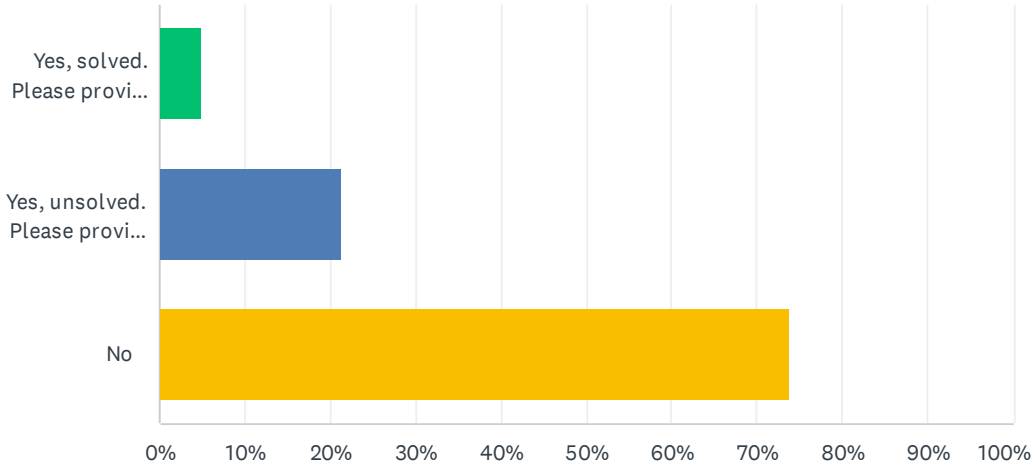


ANSWER CHOICES	RESPONSES	
CHO	86.96%	60
Other mammalian	43.48%	30
Yeast, E.Coli or other microbial	11.59%	8
Insects	5.80%	4
Fungi	2.90%	2
Plants	1.45%	1
Other (please specify)	0.00%	0
Total Respondents: 69		

#	OTHER (PLEASE SPECIFY)	DATE
	There are no responses.	

Q28 Have you faced issues with your host organism that you think/know might not be an issue with an alternative host (i.e., not CHO, HEK)?

Answered: 61 Skipped: 268



ANSWER CHOICES	RESPONSES	
Yes, solved. Please provide details in the comment box below.	4.92%	3
Yes, unsolved. Please provide details in the comment box below.	21.31%	13
No	73.77%	45
TOTAL		61

#	PLEASE PROVIDE DETAILS IF YOU ANSWER IS YES.	DATE
1	Making targeted integration for HEK cells are harder compared to CHO cells due to multiple number of copies of different choromosomes, this problem doesn't exist in CHO cells	4/13/2023 8:25 PM
2	Productivity bottleneck	4/13/2023 6:25 PM
3	challenges adapting cells out of serum is common. People want to adapt cell lines that are typically adherent to suspension	4/13/2023 3:46 PM
4	i have not issues	4/13/2023 3:03 PM
5	Speed and cost of goods.	4/7/2023 5:30 PM
6	workflow limited by doubling time; cell line limited by retrovirus history; media are not universal	4/4/2023 3:28 PM
7	PTMs, complex proteins like blood factors	4/4/2023 1:20 PM
8	Would prefer faster doubling times during seed train; genetic engineering is complex to target/modulate many genes and/or genome regions in the same host; genetic plasticity/heterogeniety	4/3/2023 7:42 PM
9	Low productivity. Difficulties to isolate good clonal cell lines without automatized systems.	4/3/2023 1:27 PM
10	Low antibody expression in CHO due to ER stress. Could be higher in e. g. glycoengineered yeast.	4/2/2023 9:44 PM
11	Challenge in optimizing production of non-protein products	4/1/2023 10:12 PM

12	Speed to market and host cell proteins.	4/1/2023 10:24 AM
13	Cell density of efficient transfection in HEK	4/1/2023 3:50 AM
14	Cell density effect during viral vector production.	3/31/2023 5:42 PM

Q29 What are some perceived challenges of working with an alternative host system?

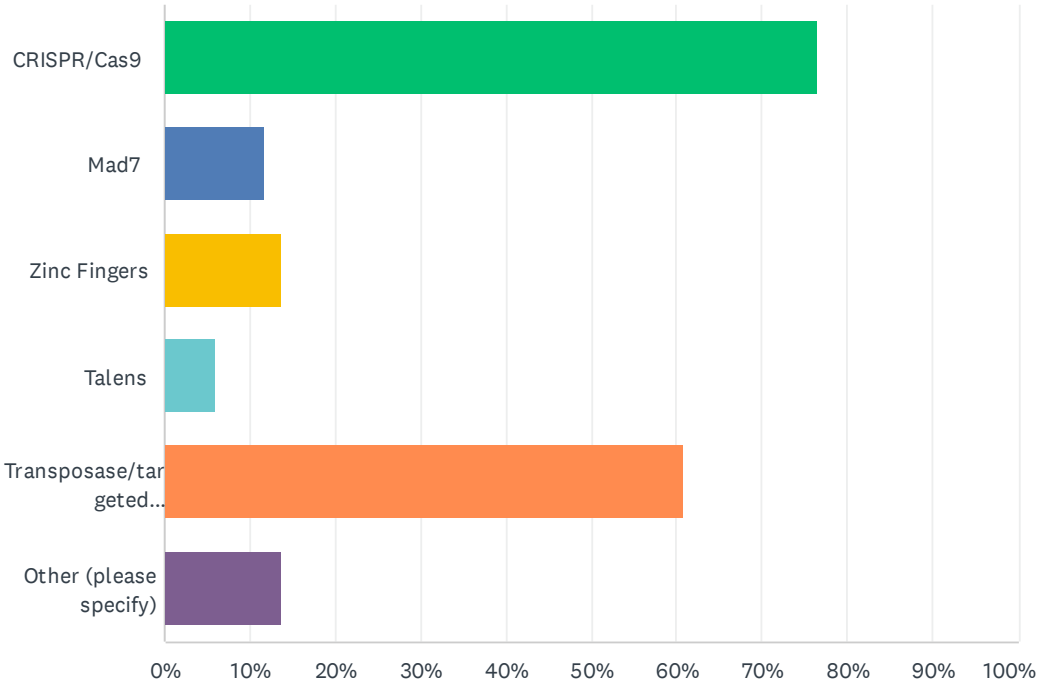
Answered: 30 Skipped: 299

#	RESPONSES	DATE
1	Need for new media and process, no historical data to rely on	4/14/2023 6:30 PM
2	regulatory acceptance, facility fit, the ability to achieve certain metrics	4/14/2023 1:53 PM
3	Proper glycosylation	4/14/2023 8:56 AM
4	Scale-ability	4/14/2023 5:58 AM
5	History of safety data, time for development and optimization, potential regulatory concerns	4/13/2023 8:36 PM
6	Final quality of the therapeutics	4/13/2023 6:25 PM
7	Regulatory, timeline for developing new techniques, transfer to manufacturing	4/13/2023 4:10 PM
8	low titer, poor product quality	4/13/2023 3:59 PM
9	less understood about metabolic pathway (or potentially more known by science at large, but less by me); current technologies geared towards most common hosts	4/13/2023 3:58 PM
10	regulatory approvals with well known cell lines. Decreased productivity but better glycosylation profiles	4/13/2023 3:46 PM
11	Glycosilation profile.	4/13/2023 3:03 PM
12	Other hosts are not established in industry -> not that interessting	4/13/2023 3:49 AM
13	There may be difficulties in the quality of the final product, also the possibility of having modifications that significantly affect the possibility of using it for a specific purpose	4/10/2023 8:27 PM
14	Low titers and change in infrastructure needed to manufacture in alt hosts.	4/7/2023 5:30 PM
15	The main characteristics could be different, e.g. the post-translational modifications or protein quantity	4/5/2023 3:29 PM
16	Lack of database information	4/5/2023 4:41 AM
17	post-translational modifications, media, regulatory agency acceptance (safety, and no one wants to be first)	4/4/2023 3:28 PM
18	New, many unknown. Regulatory prospective.	4/4/2023 2:09 PM
19	Need a new non-standard platform	4/4/2023 1:20 PM
20	new manufacturing facilities, acceptance by regulatory authorities, delay in project progression timelines due to new/unknown challenges that alternate host might present	4/4/2023 2:28 AM
21	Lack of "foundation" that we have for CHO (knowledge, media, processes, etc.)	4/3/2023 7:42 PM
22	Appropriate post-translational modifications Productivity Amenability to transfection and genetic manipulation Ease of culture	4/3/2023 11:06 AM
23	Glycoforms	4/2/2023 9:44 PM
24	Expressing complex molecules with high productivity and quality; Limited manufacturing experience and facilities	4/2/2023 10:21 AM
25	comparability in glycan structures and post-translational processing	4/2/2023 7:31 AM
26	Media design, bioprocess condition optimization	4/1/2023 10:12 PM
27	Titer, post-translational modifications, etc.	4/1/2023 10:24 AM

28	Industry acceptance.	4/1/2023 4:15 AM
29	Product quality, fit into existing workflows (cell line development equipment, manufacturing equipment), regulatory acceptance	3/31/2023 9:40 PM
30	Acceptance by industry	3/31/2023 5:01 PM

Q30 Do you work with any genome engineering systems (select all that apply)?

Answered: 51 Skipped: 278



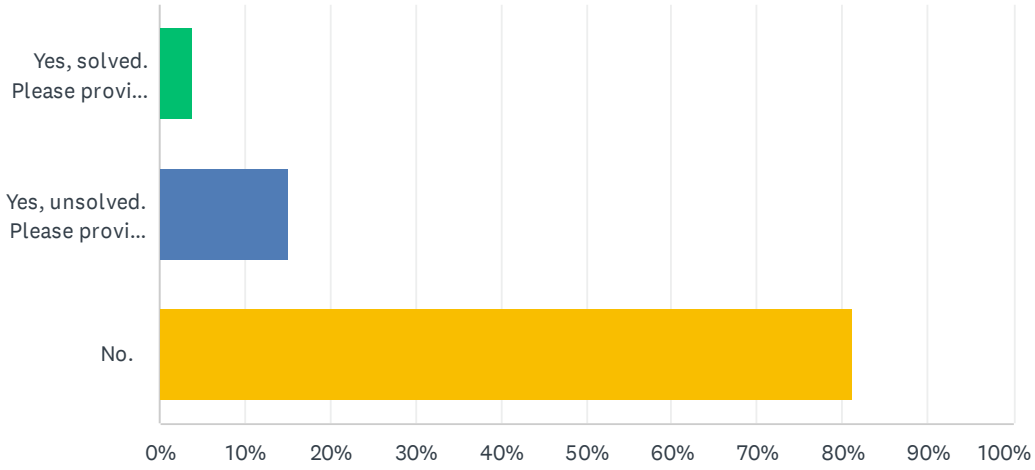
ANSWER CHOICES	RESPONSES	
CRISPR/Cas9	76.47%	39
Mad7	11.76%	6
Zinc Fingers	13.73%	7
Talens	5.88%	3
Transposase/targeted integration	60.78%	31
Other (please specify)	13.73%	7
Total Respondents: 51		

#	OTHER (PLEASE SPECIFY)	DATE
1	Recombinases (RMCE)	4/14/2023 8:56 AM
2	Recombinase	4/13/2023 3:32 PM
3	Cpf1	4/12/2023 3:45 PM
4	N/A	4/5/2023 3:29 PM
5	"Random Integration"; Plan to evaluate Cas-Clover; Considering evaluation of Mad7 - but their licensing team was very slow to reply to inquiries	4/3/2023 7:42 PM
6	Cas9 clover	4/1/2023 10:24 AM

7	Our proprietary cell engineering technologies	3/31/2023 5:01 PM

Q31 Have you faced issues with your current genome engineering system that you think might not be an issue with an alternative system?

Answered: 53 Skipped: 276

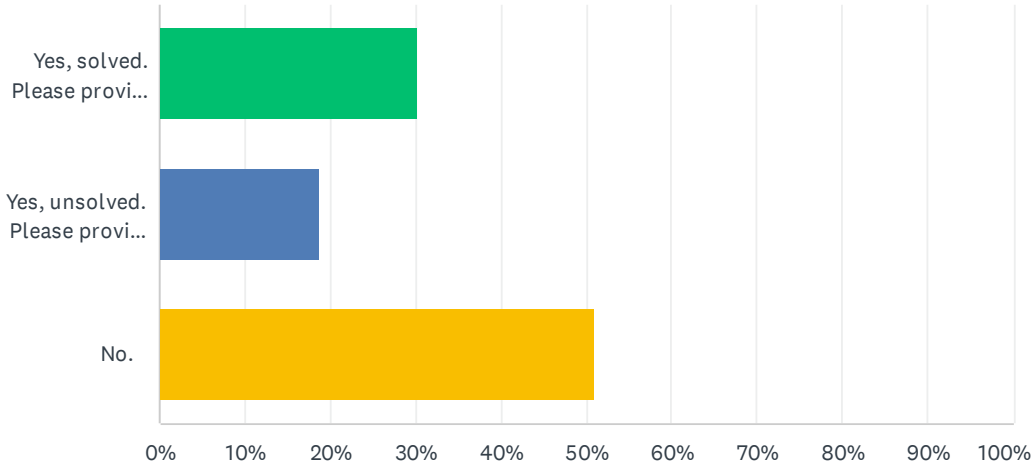


ANSWER CHOICES	RESPONSES	
Yes, solved. Please provide details in the comment box below.	3.77%	2
Yes, unsolved. Please provide details in the comment box below.	15.09%	8
No.	81.13%	43
TOTAL		53

#	PLEASE PROVIDE DETAILS IF YOUR ANSWER IS YES.	DATE
1	gene expression control and inducibility	4/13/2023 8:36 PM
2	Low efficiency of PE	4/5/2023 11:00 AM
3	random integration is time consuming; targeted takes a long time to set up properly and may not meet titer expectations, but is a difficult IP space	4/4/2023 3:28 PM
4	IP	4/4/2023 1:20 PM
5	Off-target effects; poor HDR for KIs; ZFNs take more time/$ than other approaches; common system for which research and pipeline applications can be licensed under reasonable terms; approaches to modify/remove larger genomes regions (areas of gene duplication, etc.);	4/3/2023 7:42 PM
6	DSB-mediated cytotoxicity with conventional CRISPR-Cas9 can be avoided by using alternative CRISPR technologies, such as Prime Editor.	4/3/2023 11:06 AM
7	Low recombination efficiency and thus tedious single cell sorting for stable cell line engineering. Solved through pool generation prior to sorting, however, mutations might occur.	4/2/2023 9:44 PM
8	Difficulties to identify the desired phenotypes.	4/2/2023 6:34 AM
9	Possibly less plasticity in the genome, depending on the alternate line.	4/1/2023 10:24 AM
10	Difficulties identifying the desired phenotypes.	4/1/2023 3:03 AM

Q32 Have you faced issues that you have solved or that may be solved using genome engineering?

Answered: 53 Skipped: 276



ANSWER CHOICES	RESPONSES	
Yes, solved. Please provide details in the comment box below.	30.19%	16
Yes, unsolved. Please provide details in the comment box below.	18.87%	10
No.	50.94%	27
TOTAL		53

#	PLEASE PROVIDE DETAILS IF YOUR ANSWER IS YES.	DATE
1	1) Off-target effects in many genome engineering tools 2) Product stability - lack of a universal platform 3) Medium development required for every product	4/14/2023 6:18 PM
2	Been able to knock out or knock in genes	4/14/2023 1:53 PM
3	There are a lot of improvement that could be done in order to increase product quantity and quality from genome engineering, mainly focussed on post-translation processes	4/14/2023 8:56 AM
4	Control of number of gene copies in the genome	4/13/2023 6:25 PM
5	yes and no; prototrophy and also the need to reverse reactions that are currently not reversed in cho	4/13/2023 3:58 PM
6	Improved product activity (Therapeutic enzyme), improved quality (heavy/light chain balance)	4/13/2023 3:37 PM
7	targeted takes a long time to set up properly and may not meet titer expectations, but is a difficult IP space	4/4/2023 3:28 PM
8	Knock out of problematic host cell proteins	4/4/2023 1:20 PM
9	metabolic byproduct removal, engineering cells to biosynthesize certain hard to supply nutrients.	4/4/2023 2:28 AM
10	YES - Both solved and unsolved. For example -- Modulating Glycoprofile; adding Landing Pads for TI; removing/reducing HCPs to improve purity and/or PQ; reduce unnneeded HCPs to reduce "burden" to cellular machinery during production; etc.	4/3/2023 7:42 PM

11	Genetic inactivation of endogenous retroviruses (ERVs) Site-specific integration of transgenes into pre-validated transcriptional hotspots Editing of transcriptional and metabolic pathways Identification of novel genes using CRISPR screens	4/3/2023 11:06 AM
12	Our targeted integration system ensures stable behaviour throughout prolonged time periods, which makes it possible to mix cell lines in one bioreactor for product/ antibody cocktail production.	4/2/2023 9:44 PM
13	GS knockout using ZFN	4/2/2023 10:21 AM
14	too many to list - essentially just about any engineering strategy, be it knockout or targeted integration, is more efficiently solved using these tools	4/2/2023 7:31 AM
15	Reproducibility and stability that comes with a targeted integration platform.	4/1/2023 10:24 AM
16	MULV knockout, enzyme HCP knocout, lactate phenotype	4/1/2023 10:24 AM
17	Im a synthetic biologist. Pretty much all of the problems I deal with are solved by genome engineering.	4/1/2023 4:15 AM
18	Clonal variation solved with RMCE	4/1/2023 3:50 AM
19	Unsolved: Better control of glycans levels in mAbs and complex mAbs like (Man5, sialylation, aFu, agly) Solved: Expression stability issues through GS-knockout lines and transposase integration	3/31/2023 9:40 PM
20	We have overcome inherent cellular bottlenecks to vastly higher protein productivity via CHO, or AAV productivity via HEK.	3/31/2023 5:01 PM

Q33 Please rank the following topics from 1 (Strong interest in discussion) to 10 (Don’t want to talk about):

Answered: 53 Skipped: 276

ANSWER CHOICES	RESPONSES	
Host organisms influence on product quality	94.34%	50
Host organisms influence on product quantity	96.23%	51
Host organisms influence on product purity	90.57%	48
Host organisms influence on bioprocess	94.34%	50
Comparison of genome engineering systems (e.g., maturity, IP, practicalities)	96.23%	51
Genome engineering for product quality	96.23%	51
Genome engineering for product quantity	94.34%	50
Genome engineering for product purity	92.45%	49
Genome engineering for bioprocess	96.23%	51
Other topic:	15.09%	8

#	HOST ORGANISMS INFLUENCE ON PRODUCT QUALITY	DATE
1	7	4/14/2023 6:30 PM
2	2	4/14/2023 6:18 PM
3	1	4/14/2023 5:56 PM
4	8	4/14/2023 1:53 PM
5	1	4/14/2023 9:01 AM
6	4	4/14/2023 8:56 AM
7	3	4/14/2023 5:58 AM
8	5	4/13/2023 8:36 PM
9	1	4/13/2023 8:25 PM
10	8	4/13/2023 6:25 PM
11	2	4/13/2023 4:38 PM
12	6	4/13/2023 4:10 PM
13	10	4/13/2023 3:59 PM
14	3	4/13/2023 3:58 PM
15	3	4/13/2023 3:46 PM
16	6	4/13/2023 3:37 PM
17	9	4/13/2023 3:32 PM
18	6	4/13/2023 3:49 AM
19	6	4/12/2023 3:45 PM

20	2	4/10/2023 8:27 PM
21	6	4/7/2023 5:30 PM
22	1	4/6/2023 6:58 PM
23	3	4/5/2023 3:29 PM
24	7	4/5/2023 11:00 AM
25	1	4/5/2023 4:41 AM
26	1	4/4/2023 3:28 PM
27	2	4/4/2023 2:54 PM
28	2	4/4/2023 2:09 PM
29	1	4/4/2023 1:20 PM
30	4	4/4/2023 2:28 AM
31	4	4/3/2023 9:29 PM
32	3	4/3/2023 7:42 PM
33	2	4/3/2023 1:27 PM
34	1	4/3/2023 11:06 AM
35	5	4/3/2023 4:02 AM
36	1	4/3/2023 3:42 AM
37	6	4/2/2023 9:44 PM
38	7	4/2/2023 10:21 AM
39	1	4/2/2023 6:34 AM
40	3	4/1/2023 1:08 PM
41	3	4/1/2023 10:24 AM
42	4	4/1/2023 4:15 AM
43	10	4/1/2023 3:50 AM
44	1	4/1/2023 3:03 AM
45	5	3/31/2023 9:40 PM
46	4	3/31/2023 8:32 PM
47	4	3/31/2023 5:42 PM
48	2	3/31/2023 5:11 PM
49	5	3/31/2023 5:01 PM
50	1	3/31/2023 4:58 PM

#	HOST ORGANISMS INFLUENCE ON PRODUCT QUANTITY	DATE
1	6	4/14/2023 6:30 PM
2	1	4/14/2023 6:18 PM
3	1	4/14/2023 5:56 PM
4	4	4/14/2023 1:53 PM
5	2	4/14/2023 9:01 AM
6	2	4/14/2023 8:56 AM

7	3	4/14/2023 5:58 AM
8	8	4/13/2023 8:36 PM
9	1	4/13/2023 8:25 PM
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12	7	4/13/2023 4:10 PM
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22	5	4/6/2023 6:58 PM
23	4	4/5/2023 3:29 PM
24	8	4/5/2023 11:00 AM
25	3	4/5/2023 4:41 AM
26	4	4/4/2023 3:28 PM
27	3	4/4/2023 2:54 PM
28	1	4/4/2023 2:09 PM
29	2	4/4/2023 1:20 PM
30	9	4/4/2023 2:28 AM
31	3	4/3/2023 9:29 PM
32	2	4/3/2023 7:42 PM
33	1	4/3/2023 1:27 PM
34	2	4/3/2023 11:06 AM
35	6	4/3/2023 4:02 AM
36	5	4/3/2023 3:42 AM
37	5	4/2/2023 9:44 PM
38	2	4/2/2023 11:45 AM
39	6	4/2/2023 10:21 AM
40	1	4/2/2023 6:34 AM
41	6	4/1/2023 1:08 PM
42	1	4/1/2023 10:24 AM
43	2	4/1/2023 4:15 AM
44	5	4/1/2023 3:50 AM

45	1	4/1/2023 3:03 AM
46	4	3/31/2023 9:40 PM
47	2	3/31/2023 8:32 PM
48	3	3/31/2023 5:42 PM
49	1	3/31/2023 5:11 PM
50	3	3/31/2023 5:01 PM
51	1	3/31/2023 4:58 PM
#	**HOST ORGANISMS INFLUENCE ON PRODUCT PURITY**	**DATE**
1	9	4/14/2023 6:30 PM
2	3	4/14/2023 6:18 PM
3	9	4/14/2023 1:53 PM
4	6	4/14/2023 9:01 AM
5	9	4/14/2023 8:56 AM
6	5	4/14/2023 5:58 AM
7	9	4/13/2023 8:36 PM
8	2	4/13/2023 8:25 PM
9	9	4/13/2023 6:25 PM
10	1	4/13/2023 4:38 PM
11	8	4/13/2023 4:10 PM
12	10	4/13/2023 3:59 PM
13	8	4/13/2023 3:58 PM
14	6	4/13/2023 3:46 PM
15	3	4/13/2023 3:37 PM
16	7	4/13/2023 3:32 PM
17	8	4/13/2023 3:49 AM
18	3	4/10/2023 8:27 PM
19	8	4/7/2023 5:30 PM
20	3	4/6/2023 6:58 PM
21	5	4/5/2023 3:29 PM
22	9	4/5/2023 11:00 AM
23	2	4/5/2023 4:41 AM
24	3	4/4/2023 3:28 PM
25	4	4/4/2023 2:54 PM
26	4	4/4/2023 2:09 PM
27	8	4/4/2023 1:20 PM
28	8	4/4/2023 2:28 AM
29	5	4/3/2023 9:29 PM
30	3	4/3/2023 7:42 PM

31	3	4/3/2023 1:27 PM
32	3	4/3/2023 11:06 AM
33	7	4/3/2023 4:02 AM
34	1	4/3/2023 3:42 AM
35	4	4/2/2023 9:44 PM
36	8	4/2/2023 10:21 AM
37	3	4/2/2023 6:34 AM
38	7	4/1/2023 1:08 PM
39	8	4/1/2023 10:24 AM
40	6	4/1/2023 4:15 AM
41	10	4/1/2023 3:50 AM
42	1	4/1/2023 3:03 AM
43	7	3/31/2023 9:40 PM
44	5	3/31/2023 8:32 PM
45	5	3/31/2023 5:42 PM
46	3	3/31/2023 5:11 PM
47	9	3/31/2023 5:01 PM
48	8	3/31/2023 4:58 PM

#	HOST ORGANISMS INFLUENCE ON BIOPROCESS	DATE
1	8	4/14/2023 6:30 PM
2	4	4/14/2023 6:18 PM
3	1	4/14/2023 5:56 PM
4	7	4/14/2023 1:53 PM
5	3	4/14/2023 9:01 AM
6	8	4/14/2023 8:56 AM
7	2	4/14/2023 5:58 AM
8	2	4/13/2023 8:36 PM
9	2	4/13/2023 8:25 PM
10	6	4/13/2023 6:25 PM
11	2	4/13/2023 4:38 PM
12	9	4/13/2023 4:10 PM
13	10	4/13/2023 3:59 PM
14	6	4/13/2023 3:58 PM
15	8	4/13/2023 3:46 PM
16	7	4/13/2023 3:37 PM
17	8	4/13/2023 3:32 PM
18	5	4/13/2023 3:49 AM
19	1	4/10/2023 8:27 PM

20	9	4/7/2023 5:30 PM
21	1	4/6/2023 6:58 PM
22	1	4/5/2023 3:29 PM
23	6	4/5/2023 11:00 AM
24	4	4/5/2023 4:41 AM
25	6	4/4/2023 3:28 PM
26	1	4/4/2023 2:54 PM
27	5	4/4/2023 2:09 PM
28	3	4/4/2023 1:20 PM
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30	1	4/3/2023 9:29 PM
31	4	4/3/2023 7:42 PM
32	1	4/3/2023 1:27 PM
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34	3	4/3/2023 4:02 AM
35	6	4/3/2023 3:42 AM
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42	6	4/1/2023 4:15 AM
43	5	4/1/2023 3:50 AM
44	1	4/1/2023 3:03 AM
45	6	3/31/2023 9:40 PM
46	5	3/31/2023 8:32 PM
47	1	3/31/2023 5:42 PM
48	4	3/31/2023 5:11 PM
49	4	3/31/2023 5:01 PM
50	8	3/31/2023 4:58 PM

#	COMPARISON OF GENOME ENGINEERING SYSTEMS (E.G., MATURITY, IP, PRACTICALITIES)	DATE
1	1	4/14/2023 6:30 PM
2	8	4/14/2023 6:18 PM
3	1	4/14/2023 5:56 PM
4	1	4/14/2023 1:53 PM
5	7	4/14/2023 9:01 AM
6	5	4/14/2023 8:56 AM

7	2	4/14/2023 5:58 AM
8	3	4/13/2023 8:36 PM
9	1	4/13/2023 8:25 PM
10	1	4/13/2023 6:25 PM
11	8	4/13/2023 4:38 PM
12	5	4/13/2023 4:10 PM
13	4	4/13/2023 3:59 PM
14	4	4/13/2023 3:58 PM
15	9	4/13/2023 3:46 PM
16	2	4/13/2023 3:37 PM
17	5	4/13/2023 3:32 PM
18	9	4/13/2023 3:49 AM
19	4	4/12/2023 3:45 PM
20	2	4/10/2023 8:27 PM
21	1	4/7/2023 5:30 PM
22	1	4/6/2023 6:58 PM
23	9	4/5/2023 3:29 PM
24	5	4/5/2023 11:00 AM
25	9	4/5/2023 4:41 AM
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27	5	4/4/2023 2:54 PM
28	3	4/4/2023 2:09 PM
29	4	4/4/2023 1:20 PM
30	6	4/4/2023 2:28 AM
31	8	4/3/2023 9:29 PM
32	1	4/3/2023 7:42 PM
33	4	4/3/2023 1:27 PM
34	2	4/3/2023 11:06 AM
35	8	4/3/2023 4:02 AM
36	1	4/3/2023 3:42 AM
37	9	4/2/2023 9:44 PM
38	5	4/2/2023 10:21 AM
39	2	4/2/2023 6:34 AM
40	4	4/1/2023 1:08 PM
41	1	4/1/2023 10:24 AM
42	1	4/1/2023 10:24 AM
43	4	4/1/2023 4:15 AM
44	3	4/1/2023 3:50 AM

45	1	4/1/2023 3:03 AM
46	9	3/31/2023 9:40 PM
47	2	3/31/2023 8:32 PM
48	9	3/31/2023 5:42 PM
49	9	3/31/2023 5:11 PM
50	8	3/31/2023 5:01 PM
51	1	3/31/2023 4:58 PM
#	**GENOME ENGINEERING FOR PRODUCT QUALITY**	**DATE**
1	5	4/14/2023 6:30 PM
2	4	4/14/2023 6:18 PM
3	5	4/14/2023 5:56 PM
4	2	4/14/2023 1:53 PM
5	4	4/14/2023 9:01 AM
6	3	4/14/2023 8:56 AM
7	4	4/14/2023 5:58 AM
8	4	4/13/2023 8:36 PM
9	1	4/13/2023 8:25 PM
10	5	4/13/2023 6:25 PM
11	2	4/13/2023 4:38 PM
12	1	4/13/2023 4:10 PM
13	1	4/13/2023 3:59 PM
14	1	4/13/2023 3:58 PM
15	1	4/13/2023 3:46 PM
16	1	4/13/2023 3:43 PM
17	10	4/13/2023 3:37 PM
18	4	4/13/2023 3:32 PM
19	2	4/13/2023 3:49 AM
20	3	4/12/2023 3:45 PM
21	1	4/10/2023 8:27 PM
22	2	4/7/2023 5:30 PM
23	3	4/6/2023 6:58 PM
24	7	4/5/2023 3:29 PM
25	2	4/5/2023 11:00 AM
26	5	4/5/2023 4:41 AM
27	4	4/4/2023 3:28 PM
28	7	4/4/2023 2:54 PM
29	6	4/4/2023 2:09 PM
30	6	4/4/2023 1:20 PM

31	1	4/4/2023 2:28 AM
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33	2	4/3/2023 7:42 PM
34	2	4/3/2023 1:27 PM
35	1	4/3/2023 11:06 AM
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38	8	4/2/2023 9:44 PM
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41	5	4/1/2023 1:08 PM
42	1	4/1/2023 10:24 AM
43	2	4/1/2023 4:15 AM
44	3	4/1/2023 3:50 AM
45	1	4/1/2023 3:03 AM
46	1	3/31/2023 9:40 PM
47	2	3/31/2023 8:32 PM
48	7	3/31/2023 5:42 PM
49	5	3/31/2023 5:11 PM
50	6	3/31/2023 5:01 PM
51	5	3/31/2023 4:58 PM

#	GENOME ENGINEERING FOR PRODUCT QUANTITY	DATE
1	2	4/14/2023 6:30 PM
2	1	4/14/2023 6:18 PM
3	9	4/14/2023 5:56 PM
4	3	4/14/2023 1:53 PM
5	5	4/14/2023 9:01 AM
6	1	4/14/2023 8:56 AM
7	2	4/14/2023 5:58 AM
8	6	4/13/2023 8:36 PM
9	1	4/13/2023 8:25 PM
10	2	4/13/2023 6:25 PM
11	1	4/13/2023 4:38 PM
12	3	4/13/2023 4:10 PM
13	1	4/13/2023 3:59 PM
14	2	4/13/2023 3:58 PM
15	2	4/13/2023 3:46 PM
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17	1	4/13/2023 3:32 PM
18	3	4/13/2023 3:49 AM
19	1	4/12/2023 3:45 PM
20	1	4/10/2023 8:27 PM
21	3	4/7/2023 5:30 PM
22	5	4/6/2023 6:58 PM
23	6	4/5/2023 3:29 PM
24	1	4/5/2023 11:00 AM
25	7	4/5/2023 4:41 AM
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31	7	4/3/2023 9:29 PM
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33	2	4/3/2023 1:27 PM
34	1	4/3/2023 11:06 AM
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36	3	4/3/2023 3:42 AM
37	7	4/2/2023 9:44 PM
38	1	4/2/2023 10:21 AM
39	1	4/2/2023 6:34 AM
40	1	4/1/2023 1:08 PM
41	1	4/1/2023 10:24 AM
42	2	4/1/2023 4:15 AM
43	3	4/1/2023 3:50 AM
44	1	4/1/2023 3:03 AM
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46	1	3/31/2023 8:32 PM
47	6	3/31/2023 5:42 PM
48	6	3/31/2023 5:11 PM
49	1	3/31/2023 5:01 PM
50	5	3/31/2023 4:58 PM
#	**GENOME ENGINEERING FOR PRODUCT PURITY**	**DATE**
1	4	4/14/2023 6:30 PM
2	3	4/14/2023 6:18 PM
3	5	4/14/2023 5:56 PM

4	6	4/14/2023 1:53 PM
5	8	4/14/2023 9:01 AM
6	7	4/14/2023 8:56 AM
7	3	4/14/2023 5:58 AM
8	7	4/13/2023 8:36 PM
9	1	4/13/2023 8:25 PM
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11	2	4/13/2023 4:38 PM
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13	1	4/13/2023 3:59 PM
14	9	4/13/2023 3:58 PM
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25	5	4/4/2023 3:28 PM
26	9	4/4/2023 2:54 PM
27	8	4/4/2023 2:09 PM
28	9	4/4/2023 1:20 PM
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30	9	4/3/2023 9:29 PM
31	2	4/3/2023 7:42 PM
32	2	4/3/2023 1:27 PM
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38	2	4/2/2023 6:34 AM
39	9	4/1/2023 1:08 PM
40	8	4/1/2023 10:24 AM
41	6	4/1/2023 4:15 AM

42	5	4/1/2023 3:50 AM
43	2	4/1/2023 3:03 AM
44	8	3/31/2023 9:40 PM
45	4	3/31/2023 8:32 PM
46	8	3/31/2023 5:42 PM
47	7	3/31/2023 5:11 PM
48	7	3/31/2023 5:01 PM
49	5	3/31/2023 4:58 PM
#	**GENOME ENGINEERING FOR BIOPROCESS**	**DATE**
1	3	4/14/2023 6:30 PM
2	3	4/14/2023 5:56 PM
3	5	4/14/2023 1:53 PM
4	9	4/14/2023 9:01 AM
5	6	4/14/2023 8:56 AM
6	3	4/14/2023 5:58 AM
7	1	4/13/2023 8:36 PM
8	2	4/13/2023 8:25 PM
9	3	4/13/2023 6:25 PM
10	2	4/13/2023 4:38 PM
11	4	4/13/2023 4:10 PM
12	1	4/13/2023 3:59 PM
13	5	4/13/2023 3:58 PM
14	7	4/13/2023 3:46 PM
15	2	4/13/2023 3:43 PM
16	8	4/13/2023 3:37 PM
17	3	4/13/2023 3:32 PM
18	1	4/13/2023 3:49 AM
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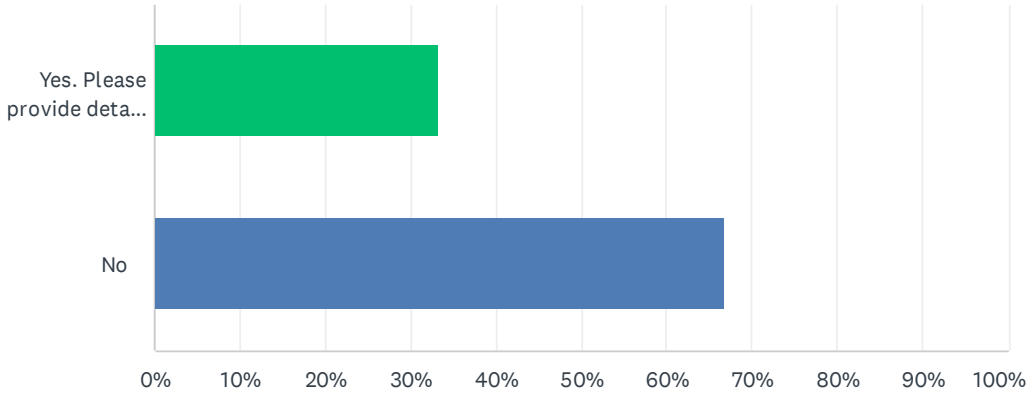
44	3	4/1/2023 3:50 AM
45	1	4/1/2023 3:03 AM
46	3	3/31/2023 9:40 PM
47	7	3/31/2023 8:32 PM
48	2	3/31/2023 5:42 PM
49	8	3/31/2023 5:11 PM
50	2	3/31/2023 5:01 PM
51	1	3/31/2023 4:58 PM

#	OTHER TOPIC:	DATE
1	10	4/13/2023 4:10 PM
2	1	4/13/2023 3:37 PM
3	10	4/13/2023 3:32 PM
4	10	4/4/2023 1:20 PM
5	systems biology; targeting many genes and/or larger genome regions; importance of transcript / protein evaluation (splicing, etc); challenges with hamster reagents	4/3/2023 7:42 PM
6	10	4/2/2023 9:44 PM
7	10	4/2/2023 10:21 AM
8	10	3/31/2023 9:40 PM

Q34 Have intellectual property concerns stopped you from using novel technologies?

Answered: 57 Skipped: 272



ANSWER CHOICES	RESPONSES	
Yes. Please provide details below.	33.33%	19
No	66.67%	38
TOTAL		57

#	PLEASE PROVIDE DETAILS IF YOUR ANSWER IS YES.	DATE
1	IP around CRISPR makes it challenging to use even for research use.	4/14/2023 6:30 PM
2	CRISPR editing technologies for host cell line engineering still remains as a huge bottleneck for many, pushing many to the sideline.	4/14/2023 6:18 PM
3	crispr patent landscape	4/13/2023 8:36 PM
4	CRISPR	4/13/2023 4:10 PM
5	originally some concern over using crispr, but used in a non-commercial space	4/13/2023 3:58 PM
6	IP stops developers from being dual sourced if needed. More and more industry leaders want more control over IP to have more flexibility in their supply chain	4/13/2023 3:46 PM
7	transposases and crispr Cas9	4/13/2023 3:37 PM
8	Cas9 IP ambiguity	4/12/2023 3:45 PM
9	Genome editing technologies FTO confusion	4/7/2023 5:30 PM
10	Costs are a big concern	4/4/2023 1:20 PM
11	Use for pipeline CLD requires costly licensing	4/3/2023 7:42 PM
12	CRISPR; certain transposases These IP restrictions can cause innovation bottlenecks.	4/3/2023 11:06 AM
13	CRISPR	4/3/2023 3:42 AM
14	Messy CRISPR patent landscape, especially for commercial use	4/2/2023 10:21 AM
15	CRISPR IP situation	4/1/2023 10:24 AM
16	But I don't understand the feedback I get from companies about IP. I've heard conflicting things	4/1/2023 4:15 AM

	- that companies want to work with patented technologies they can license vs. wanting to avoid working with patented technologies.	

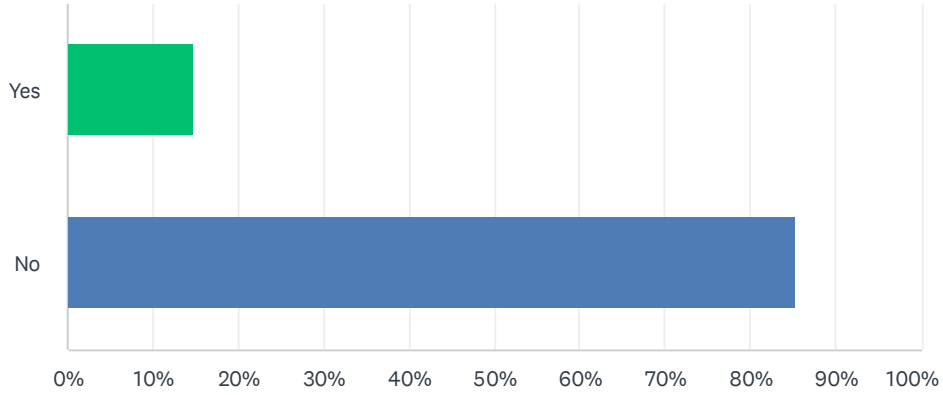
Q35 Are there particular case studies you would like to discuss? Please describe in detail.

Answered: 6 Skipped: 323

#	RESPONSES	DATE
1	No	4/14/2023 6:18 PM
2	Off-target safety issues	4/14/2023 5:56 PM
3	No	4/13/2023 3:49 AM
4	NO.	4/10/2023 8:27 PM
5	I'd like to see if anyone has used Prime Editing for precision editing in CHO or other systems.	4/5/2023 11:00 AM
6	N/A	4/1/2023 10:24 AM

Q36 Would you be willing to do a 5-minute presentation?

Answered: 54 Skipped: 275



ANSWER CHOICES	RESPONSES	
Yes	14.81%	8
No	85.19%	46
TOTAL		54

#	PLEASE PROVIDE YOUR NAME AND EMAIL ADDRESS IF YOUR ANSWER IS YES.	DATE
1	Zhimei Du, zhimeidu@gmail.com	4/14/2023 5:56 PM
2	nsandova@tulane.edu	4/5/2023 11:00 AM
3	Bhanu Chandra Mulukutla, bhanuchandra.mulukutla@pfizer.com. I can try, need to get approval for presenting research	4/4/2023 2:28 AM
4	shaida.x.moghaddassi@gsk.com	4/3/2023 11:06 AM
5	james.ravellette@milliporesigma.com, James Ravellette	4/1/2023 10:24 AM
6	gbolton@amgen.com	4/1/2023 10:24 AM
7	Larry Forman - larry@cho-plus.com	3/31/2023 5:01 PM