TECHNICAL TRANSFER AT CULTURE BIOSCIENCES: ENSURING SUCCESSFUL SCALE-DOWN OF CLIENT PROCESSES

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Summary

Instead of individual companies establishing their own bioreactors in-house, Culture Biosciences enables all the advantages of additional lab capacity with a streamlined process. To ensure compatibility and to maximize the success of the specified work, Culture Biosciences has implemented a technical transfer process with built-in acceptance criteria tailored to the needs of each client. These acceptance criteria describe experimental results that must be attained before advancing to the ongoing capacity portion of the contract, giving clients confidence in the scale-down model that is generated at Culture before embarking on work specified in the contract. This framework facilitates a technical transfer process with clear project objectives and communication throughout the process, and results in successful downscaling of client bioprocesses.

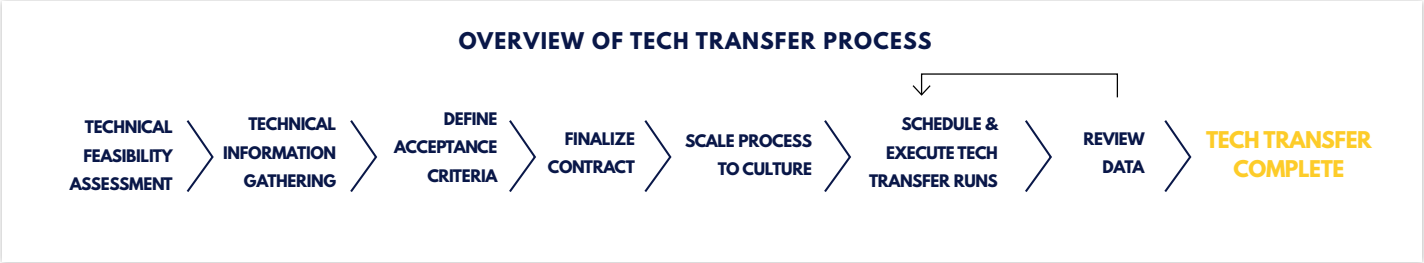
IMPORTANCE OF TECHNICAL TRANSFER

Working with Culture Biosciences allows clients to access bioreactor capacity as either their only source of bioreactors or to supplement their internal capacity. In either scenario, it is imperative that the results obtained at Culture translate to other facilities as clients scale their processes. Smaller scale bioreactors have the advantage of decreasing cost and increasing the number of tests that can be run in parallel. However, most clients face the barrier of accurately scaling down the reaction in a representative way. Challenges with scalability are common in bioprocessing, because work is transferred to different reactors and facilities as projects advance towards commercialization. Without thoroughly characterizing reactor systems and heightened attention to detail throughout the technical transfer process, variables important to strain performance can be overlooked resulting in failure to replicate performance at different scales. Culture's technical capabilities and expertise provide clients with the support necessary to overcome these challenges.

Accordingly, in addition to providing operational excellence and high quality data, Culture Biosciences has invested in thorough characterization of their 250mL cloud bioreactors and developed a framework to facilitate successful downscaling of procedures from client sites. This framework ensures that a successful technology transfer is completed prior to embarking on the guaranteed capacity portion of a project. Together, these efforts allow work performed at Culture to be rapidly and accurately translated to work being performed at other facilities.

Here, we describe the stages of the technical transfer process when working with Culture, and present examples of how this framework facilitates successful technical transfer and downscaling of bioprocesses.

STAGES OF TECHNICAL TRANSFER



TECHNICAL FEASIBILITY ASSESSMENT

The Culture team begins working with potential clients by understanding the technical suitability of the proposed work. This may include discussions around the organism being used, goals of the work to be performed at Culture, the technical specifications of the system being transferred from, or an assessment of material compatibility. Once a project has been ascertained to be well suited to Culture's capabilities, the formal technical transfer process is initiated.

TECHNICAL INFORMATION GATHERING

Ensuring work can be supported by Culture's capabilities and identifying areas for advancement

Culture Biosciences has experience transferring processes with different organisms and across the spectrum of developmental stages. Examples include transferring shake flask cultivations, setting up initial bioreactor conditions, screening strains, developing processes to support more mature programs, and running experiments to help improve processes already running at industrial scale.

Given the wide variety of projects supported by Culture Biosciences, the technical transfer process begins with a period of technical information gathering. This is to ensure that the technical specifications of Culture's bioreactors and other capabilities (such as available assays) are well suited for the project and that the process design can be defined according to the client's reactor specifications. In addition, this step allows the Culture team to determine what aspects of the proposed work may be enhanced by Culture's cloud bioreactors' features, such as the capabilities to deliver multiple feeds, perform mass based measurements, and execute sophisticated control recipes by leveraging online data signals.

Technical details collected include a process description with control recipes, example run data, equipment specifications, and procedures for sample processing and analysis. This activity is facilitated by the use of detailed templates and a dedicated Bioprocess Alliance Management team, as well as experienced Technical Transfer Bioprocess Engineers experienced in technical transfer of bioprocesses. This simplifies the procedure for the client, as it provides guidance for communicating the relevant information required for a successful technical transfer process. It also minimizes the chances of missing information or miscommunications, which can jeopardize the success of the entire technical transfer process.

After review of initial documentation, the Culture team collaborates with the client's bioprocessing representatives to understand nuances in the client's bioprocess such as observations to be aware of, typical failure modes, and what progress the client is aiming to achieve by working with Culture. This additional context prepares the Culture team to troubleshoot potential issues, build features to leverage physiology within the process, and avoid common failure modes.

ACCEPTANCE CRITERIA

A quantitative framework for assessing technical transfer

As mentioned above, in line with these diverse technical projects, the specific goals and experimental work for each project can be similarly diverse. To achieve confidence in the translatability of the down-scaled model, setting clear objectives ahead of project work is critical to project success when working with an external bioprocessing facility. In appreciation of this, Culture has developed a framework of acceptance criteria for technical transfer that is tailored to the individual specifications and goals of a project. Within this framework, clients decide on a set of conditions defined by available bioprocess data that are to be met during technical transfer before moving to the next phase of the project. The overall goal of technical transfer is to generate confidence in the translatability of the scale down model. However, due to almost universally tight project timelines, the stringency of technical transfer must be balanced with the time and resources required to complete this phase of the project. This is where the acceptance criteria framework of technical transfer can guide clients to select the most appropriate metrics to ensure project success. Stringency is applied to the most relevant metrics based on existing available client data and specific project goals, allowing clients to strike a balance between the goals of the project and meeting strict timelines.

Acceptance criteria are divided into 3 categories:

PROCESS CONTROL

Process control criteria determine how tightly process setpoints should be controlled within the bioprocess. Specified process control criteria dictate how closely a particular parameter must be controlled around the setpoint.

INTERVAL AND ENDPOINT METRICS

The ability to reproduce strain performance is critical to the successful transfer of any bioprocess. Interval and endpoint metrics enable the client to define aspects of the process that are critical to replicate in order to demonstrate confidence in the technical transfer of the bioprocess.

REPRODUCIBILITY

In order to make statistically sound conclusions from fermentation data, it is necessary to reproduce results within a defined range, which is dependent on both the stage of the project and any existing client data. Reproducibility criteria can define the level of acceptable variation.

How acceptance criteria are formulated

The specific details of the acceptance criteria are generated based on existing client data. This is individualized for each client and dependent on the project, the stage of the program, the goals of the work, and the reason acceptance criteria are formulated. The requirements for a process being transferred to a bioreactor from a shake flask for the first time may be vastly different from a bioprocess being refined through process development or a project focused on strain screening within a single process. Thus, this framework was developed with flexibility in mind to address specific clients' project goals. As can be seen in Table 1, this flexibility is reflected in the specific acceptance criteria that may be implemented for technical transfer of an individual project.

This framework allows clients to gain confidence in the developed scale-down model and complete successful technical transfer with stringency and timelines appropriate for the individual project, despite the diversity in goals and stages of different projects.

PROJECT	AIM OF WORK	FOCUS OF TECH TRANSFER	EXAMPLE ACCEPTANCE CRITERIA
Process Setup	Transfer process from shake flask and develop bioreactor process	Demonstrate process control and growth of the organism without contamination	**Process setpoints:** Control pH within +/- 0.1 Demonstrate growth without contamination
Strain Screening	Screen different strains for improved performance	Demonstrate reproducible performance of a control strain and accurate ranking of strains with different performance	**Process setpoints:** Control pH within +/- 0.1 Demonstrate final titer of 10g/L +/- 0.5 g/L in 3 replicates in 2 distinct runs Demonstrate %CV <5% within run and %CV <7.5% between runs
Process Development	Perturb the process to look for improved performance	Establish baseline performance with a single strain and demonstrate impact of varying a factor with a known income	**Process setpoints:** Control pH within +/- 0.1 Demonstrate final titer of 10g/L +/- 0.5 g/L in 3 replicates in 2 distinct runs Demonstrate 5C decrease in temperature results in 20% decrease in titer

Table 1: *Example technical transfer acceptance criteria for client projects at different stages.*

FINALIZE CONTRACT

The acceptance criteria are set before finalizing the statement of work (SOW) between a client and Culture Biosciences. By doing this, there is a go/no-go decision contingent on fulfillment of the acceptance criteria specified in the SOW prior to moving onto the guaranteed capacity portion of the contract. This allows clients to have confidence that the acceptance criteria and required bioprocess performance will be met prior to moving to the next stage of the contract. The technical transfer process is also structured as a fixed-fee piece of work, which ensures that experiments will be performed as necessary in order to fulfill the criteria and not limited to a predefined number of runs. This allows for the flexibility to iterate as necessary until the technical transfer acceptance criteria are met.

SCALE PROCESS TO CULTURE

Clients contract with Culture to transfer bioreactor capacity from a wide variety of vessel configurations and bioreactor scales. Successful bioprocess transfer and scale-down requires matching critical process parameters. Technical familiarity of the bioreactors is necessary for matching process parameters across sites and an understanding of what factors may need to be optimized in technical transfer. To accomplish this, Culture has invested in a thorough characterization of their bioreactors. Culture's reactors are versatile - equipped with up to five feeds, each delivered with scaled feedback control, the option for oxygen supplementation, and continuous offgas measurements that can be used for process control; they offer the flexibility to execute a wide variety of bioprocesses. There are also options for customization available, such as customized impeller designs to match client specifications.

Historically, technical transfer and scale down of bioprocesses too often faced obstacles. There are many factors that can impact bioprocess performance, and it can be challenging to pinpoint factors responsible for a failure to match performance across sites. In short, every process detail is important in the context of technical transfer and failure to precisely translate all parameters across sites can result in unnecessarily lengthy technical transfers (Table 2). With this in mind, Culture's team has developed a technical transfer package designed to facilitate communication between teams, minimize the frequency of missed details, and account for the nuances in procedures that can impact bioprocess performance.

COMMON TECHNICAL TRANSFER FAILURE MODES

Failure Mode	Culture's Process
Media	Culture uses the same media components as the client (vendors or lot numbers if required). If media formulation is complex, prepared media can be shipped from the client during technical transfer for direct comparison against media prepared at culture.
Seed train	Culture uses seed vials generated by the client in order to reduce variability. Flask types (ie. baffled vs. non-baffled) are matched across sites, and shaker agitation settings are adjusted to match kLa between sites.
Bioprocess recipe/scripting	The technical transfer process involves collaboration between client representatives and Culture's Bioprocess Alliance Management team and bioprocess engineers in order to gain a thorough understanding of the bioprocess, as well as the logic supporting it. This communication is facilitated by the use of templates to ensure all process details are captured. Culture's bioprocess engineers are experienced with Culture's recipe scripting system and develop the logic for each bioprocess. Nonetheless, each recipe is simulated before being implemented into production to ensure the logic is executed as intended.
Bioreactor setpoints	Culture has thoroughly characterized their reactors, which allows bioreactor setpoints, such as tip speed or mass transfer to be translated from different client systems based on empirical data. Additionally, if the client reactors (or planned scale-up reactors) have limitations, such as a ceiling on oxygen transfer or feed rates, these limitations can be built into the scale-down model accordingly. Culture's reactors have features that allow flexibility in process design, such as the ability to feed *5* independent feeds or supplement oxygen to the bioreactor. This allows processes to be transferred successfully from a wide range of client reactor configurations. Culture's reactors have precision control features that enable tightly controlled setpoints. These include mass based feed control, gases delivered via mass flow controllers and a variety of available online measurements that can be used for automated feed control.
Assays/ instrumentation	Any assays transferred may also be subjected to a similar set of acceptance criteria as the bioprocess itself. Where applicable, calibration of instruments across sites using prepared standards can help facilitate this process.

Table 2: *Common challenges of technical transfers.*

The process is initiated with a predefined template for technical transfer, where the relevant process details such as media, seed train, and bioreactor information are filled in. This helps to ensure that no process details are overlooked in documentation, and reduces the chance of miscommunications that may jeopardize a successful transfer. Culture recognizes the importance of open communication between teams and takes proactive steps to achieve this. Culture's team reviews the completed template and works with the client to establish initial bioprocess conditions and to understand nuances of the process that have the potential to impact performance. Throughout the process, Culture captures potential sources of variation across sites so that they can be minimized or eliminated entirely. For example, Culture will utilize media components from the same vendors as the clients to minimize the chance of media components causing differences in the bioprocess performance. Additionally, Culture will use seed vials prepared and validated by the client to minimize variation in the seed train. This level of attention to detail is essential for successful technical transfers.

SCHEDULE AND EXECUTE TECHNICAL TRANSFER RUNS

Once the initial process parameters have been defined, runs are scheduled and executed. Depending on the length and complexity of the process, as well as the amount of available supporting data, initial technical transfer experiments may target the entire process or individual components of the process to be optimized sequentially. For example, an initial run with a new organism or a lengthy process may be necessary to optimize the batch phase in a curtailed experiment in order to minimize turnaround time in the event of the need for optimization in the initial stages of the bioprocess.

Similarly, technical transfer of an early stage process that has not yet been performed in bioreactors before may comprise two stages; one shake flasks stage where process performance is matched across sites followed by a bioreactor stage where the acceptance criteria are focused primarily on process control.

While initial experiments usually closely replicate the performance in client bioreactors due to readily available data on bioreactor specifications, the need for iteration to closely match observed strain physiology and performance is often required. These iterations may include making adjustments to reactor setpoints guided by the pilot technical transfer experiment. The technical transfer stage is a fixed fee piece of work; thus no additional costs in the development of a client scale-down bioprocess are passed along to the client when several iterations on the process are required. This ensures that the scale-down model developed for the client is of the highest quality and removes concerns about unexpected fees being incurred as a consequence of additional runs performed to improve the quality of the scale-down model.

During the technical transfer stage, iterations are performed until the acceptance criteria defined in the statement of work are fulfilled. This is a collaborative exercise where Culture's team will review data with clients, when necessary, to identify the root causes for any differences in strain physiology. Culture's team draws upon both their experience and any insights from clients during data reviews to determine the next step. Culture's team will then design experiments to test conditions that are anticipated to more closely match strain performance between sites. This process continues until all acceptance criteria have been met. The length of time required to complete technical transfer runs is dependent on the turnaround time on sample analysis, but runs can be scheduled sequentially in order to minimize the total amount of time required to complete technical transfer.

Below are some use cases demonstrating the progression of technical transfer and fulfillment of acceptance criteria in the context of different client projects with distinct requirements, supporting material, and need for iterative adjustments to the process.

USE CASE 1 - INITIAL PROCESS TRANSFER

Like a number of Culture's clients, this client uses Culture's reactors as their sole bioreactor capacity. Many companies, such as those that have raised early funding rounds, choose not to build out bioprocess labs and the associated teams required to run them as the capital equipment is expensive and requires specialized expertise in order to operate. Instead, they partner with Culture in order to allow their scientists to dedicate their focus on developing robust bioprocesses rather than the logistical tasks associated with running a physical bioreactor lab and managing all of the data streams. Therefore, client data was based on preliminary shake flask culture data at the time of tech transfer. The shake flask data were collected on a single, early stage, production strain over the course of several experiments.

Goals of work with Culture:

Develop a baseline process, screen strains, and further develop the bioprocess to maximize strain performance and scalability. To increase performance by identifying beneficial genetic edits, screening strains with a bioprocess with low variability is important in order to reliably identify improved strains.

Important points to demonstrate in technical transfer:

Robust process control in a baseline process and reproducible results between weeks.

Acceptance criteria:

Process control:
Temperature +/- 0.5 of setpoint, pH +/- 0.1 of setpoint, maintain %DO +/- 5% of setpoint, once it is reached.

Mid and endpoint metrics:
N/A, as no client supporting data is available.

Reproducibility:
% CV of control biomass and titer over 3 consecutive runs used as control metrics for future runs; if control strain variability exceeds this CV, then run fails variability metrics and will be repeated.

Results:

Process control:
Process control criteria were met across all experiments, as the defined process control parameters were well within the specifications of Culture's bioreactors.

Reproducibility:
Data was collected from four consecutive experiments using a control strain. This data was used to calculate variability metrics, both within and between experiments. These metrics were subsequently used in future experiments.The variability of the control strain titer or a WCW higher than observed in the technical transfer phase are the basis for an operational QC failure of a run for the remainder of the contract.

Based on the variability observed in the technical transfer phase, variability targets were set at 10.5% and 12.5% CVs for titers of the control strain within and between runs, respectively. Similarly, the requisite variability for maximum WCW values of the control strain during the guaranteed capacity experiments were 3.25% and 3.5% for within and between experiments, respectively.

Total runs required for technical transfer:

As this process was relatively straightforward, and there was no existing process data specified. A pilot run with two different agitation/aeration cascades was run before selecting the one that fit the desired biological outcome for the next three experiments.

Experiment	Number of Reactors	Conditions	Process Control	Interval/ Endpoint Metrics	Reproducibility
1	6	Aeration/agitation cascade A	All defined process parameters met	N/A	Data collection in progress
2	3	Aeration/agitation cascade B	All defined process parameters met	N/A	Data collection in progress
3	3	Triplicate of aeration/agitation cascade A	All defined process parameters met	N/A	Data collection in progress
Total Technical Transfer Runs	12			Not applicable as there was no process data	**Data collected from 3 experiments:** Titer CV within expt: 10.5% Titer CV between expt: 12.5% WCW CV within expt: 3.25% WCW CV between expt: 3.5%

Table 3: *Total runs required for technical transfer.*

USE CASE 2 - BASELINE BIOREACTOR PROCESS TRANSFER

This client has limited internal bioreactor capacity and has developed multiple bioreactor processes internally, but does not have the capacity to perform required process development and strain screening work. The client has process data for four different strains, each of which has been tested in three different processes. This includes reproducibility data, where Culture's targeted variability was set to match or improve variability observed at client's site.

Goals of work with Culture:

The client intends to incorporate both strain screening and process development work into their work at Culture, and the project is still in relatively early stages. The timelines for this project are challenging and require rapid progress, which in turn requires access to a significant amount of bioreactor capacity.

Important points to demonstrate in technical transfer:

Accurate process control, assessment of strain performance and impact of process setpoints.

Acceptance criteria:

Based on the amount of available data and the types of work to be performed on this project, acceptance criteria were selected to test both relative strain performance and the impact of changing process parameters in Culture's scale-down model. However, project timelines and project stage made it impractical to test conditions based on all of the available data. Accordingly, the scope of technical transfer was limited to accurately assessing strain performance of three strains in a single process, and the performance of one of these strains in two of the available bioprocesses. This enabled the scope of the technical transfer work to both have the stringency required to validate the scaled-down bioprocess and be limited enough in order to be completed in a timely manner.

Process control: Temperature +/- 0.5 of setpoint, pH +/- 0.1 of setpoint, maintain %DO +/- 5% of setpoint, once it is reached.
Mid and endpoint metrics:

Process 1:

Final titer Strain A: 8 g/L +/- 0.4 g/L
Final titer Strain B: 9 g/L +/- 0.5 g/L
Final titer Strain C: 10.5 g/L +/- 0.6 g/L

Final biomass Strain A: 107 g/L +/- 5.4 g/L
Final biomass Strain B: 106.3 g/L +/- 5.5 g/L
Final biomass Strain C: 102.5 g/L +/- 4.8 g/L

Process 2:

Final titer Strain C: 9.3 g/L +/- 0.5 g/L

Final biomass Strain C: 104.7 +/- 6.1 g/L

Reproducibility:

The client did not routinely collect variability data on their process as it was in early development stages and large increases in performance were anticipated to minimize the importance of process variability during this stage of the project. Reproducibility will be targeted as a metric after some initial process development and strain screening result in improved strain performance.

Total runs required for technical transfer:

For this program, several feed triggers were designed for the initial technical transfer experiment, using different online signals; pH, DO and a combined DO and carbon dioxide evolution rate (CER). The one that gave the most robust signal, with the combination of an increase in %DO and decrease in CER, was selected for all subsequent experiments.

After the initial experiment, all strains were tested in the baseline process and the lead strain from the baseline process was then tested in the second process in order to fulfill the acceptance criteria.

Experiment	Number of Bioreactors	Conditions	Process Control	Interval/ Endpoint Metrics	Reproducibility
1	6	**Strain C:** feed trigger design 1, process 1 **Strain C:** feed trigger design 2, process 1 **Strain C:** feed trigger design 3, process 1 Feed trigger design 3 selected based on robust signal (will be used in all subsequent runs)	All defined process parameters met	Testing of initial feed triggers. Will require optimization of trigger magnitude.	N/A
2	12	**Strain A:** process 1 **Strain B:** process 1 **Strain C:** process 1 **Strain C:** process 2	All defined process parameters met	Process metrics met	N/A
3	3	**Strain C:** process 1	All defined process parameters met	Process metrics met	N/A
Total Technical Transfer Runs	21		Yes	Process metrics met	

Table 4: *Total runs required for technical transfer.*

USE CASE 3 - ESTABLISHED PLATFORM PROCESS TRANSFER

In this example, the client has an established platform bioprocess for the production of their product. The client has a large amount of historical Chinese Hamster Ovary (CHO) clone performance data from this platform bioprocess for different products. For the product of interest in this project, the client has performance data on six different clones.

Goals of work with Culture:

The scope of the work with Culture is primarily clone screening in a well-established platform process. A number of clones will be screened for improved performance in this process. Several different products may be evaluated using Culture's bioreactor capacity.

Important points to demonstrate in technical transfer:

Accurate process control in a platform process, reproducible results between weeks.

Acceptance criteria:

Given that the scope of this work is exclusively focused on evaluating different clones for performance, it is important that strain performance is accurately assessed at Culture. Given this, acceptance criteria were developed to primarily assess accuracy of strain performance.

Process control: Temperature +/- 0.2 of setpoint, pH +/- 0.1 of setpoint, maintain %DO +/- 5% of setpoint, once it is reached.

Mid and endpoint metrics:
Final titer of four clones within +/- 10% observed and ranked the same as at the client site.
Maximum viable cell density (VCD) observed within +/- 12 hours as observed at the client site.

Reproducibility: The reproducibility targets were set to match internal client variability data provided to Culture during process review. Within an experiment, the target was CV <8%, between experiments, the target was CV <12% for control strain in the platform "control" process.

Total runs required for technical transfer:

This program consisted of a platform process and a time-based bolus feeding profile. Given the relative simplicity of the process from a control perspective, a single condition was tested in the pilot run. However, the dissolved oxygen in the pilot run descended to the setpoint more rapidly than anticipated, indicating that oxygen transfer rates were likely different between systems, as the client had not fully characterized oxygen transfer in their system. An additional experiment was performed with an altered initial agitation and aeration setpoints that resulted in dissolved oxygen trends that more closely matched those at the client site.

Subsequent experiments with these conditions were used to gather variability data on the control clone and for ranking of titer data from the four different clones included in the acceptance criteria.

Experiment	Number of Bioreactors	Conditions	Process Control	Interval/ Endpoint Metrics	Reproducibility
1	3	**Clone 1:** control process	All defined process parameters met	Dissolved oxygen trends did not match client's	
2	9	**Clone 1:** increased initial agitation **Clone 1:** increased initial aeration **Clone 1:** increased initial agitation and aeration The condition where both the initial agitation and aeration were increased resulted in dissolved oxygen trends that closely matched the client's. This condition was used for remaining experiments.	All defined process parameters met	Testing different conditions. Process metrics met with one of the conditions	Data collection in progress
3	3	**Clone 1:** updated process	All defined process parameters met	Process metrics met	Data collection in progress
4	12	**Clone 1:** updated process **Clone 2:** updated process **Clone 3:** updated process **Clone 4:** updated process	All defined process parameters met	Process metrics met	Data collection in progress
Total Technical Transfer Runs	27		All defined process parameters met	Process metrics met Titer of 4 clones within 10% of and ranked the same as observed at client site Maximum VCD with 12 hours of time observed at client site	Titer CV within expt: 7.25% Titer CV between expt: 10.5%

Table 5: *Total runs required for technical transfer.*

USE CASE 4 - ESTABLISHED PROCESS CHARACTERIZATION

In this example, the client aimed for commercial scale-up of their process. Accordingly, they had a large amount of data on their lead strain in an optimized bioprocess. They also had some historical data around the impact of changing process setpoints on strain performance.

Goals of work with Culture:

For this project, the client was primarily interested in process characterization to de-risk scale-up activities. This work involved a characterization of the process, as well as testing the impact of several conditions that may be encountered at scale, such as oscillations in substrate concentration by pulse feeding.

Important points to demonstrate in technical transfer:

Accurate process control and strain performance assessment of the lead strain, as well as replicating the impact of changing a process setpoint.

Acceptance criteria:

As there was a large amount of data available around the lead strain in the optimized process, and the goal of the work was primarily to understand the impact of process perturbations on facets of strain performance, acceptance criteria were focused on replicating the performance of the lead strain.

Process control: Temperature +/- 0.2 of setpoint, pH +/- 0.1 of setpoint, maintain %DO +/- 5% of setpoint, once it is reached.

Mid and endpoint metrics:

Current process:

Fermentation batch time:
+/- 1 hour of client site observations.

Final titer of strain:
+/- 5% of client site observations.

Final yield of strain:
+/- 3% of client site observations.

Maximum OD600:
+/- 5% of client site observations.

Historical process decreases titer by 15%:
(an older process that was optimized to improve performance)

Fermentation batch time:
+/- 1 hour of client site observations.

Final titer of strain:
+/- 5% of client site observations.

Final yield of strain:
+/- 3% of client site observations.

Maximum OD_{600}:
+/- 5% of client site observations.

Reproducibility: The reproducibility targets were set to match internal client variability data provided to Culture during process review. Within an experiment the target was CV <4%, between experiments, the target was CV <6%.

Total runs required for technical transfer:

This client aimed to perform process characterization and perturbation in preparation for scale-up of a bioprocess. The client was able to provide a relatively large dataset on the production strain in a control process. This process was transferred with metrics that were observed at the client site. Once the dissolved oxygen reached maximum agitation and aeration in the initial experiment, the growth rate was noted to be slightly higher than in the client reactors, resulting in slightly higher productivity during the process. In comparison to the client bench scale reactors, which were not outfitted with offgas capabilities, the measured oxygen uptake rates (OURs) were higher than anticipated. While kLa studies had been performed in the client reactors, the OURs observed were slightly higher than desired in Culture's reactors. Accordingly, the maximum oxygen transfer rates were limited empirically in a subsequent experiment in order to match the physiology observed at the client site and eventually at scale. This adjustment successfully limited the oxygen transfer rates in Culture's reactors and resulted in performance matched across sites.

The following experiments were used to gather process and variability data, as well as to demonstrate the impact of running the strain in a historical process in order to fulfill the acceptance criteria and complete technical transfer.

Experiment	Number of Bioreactors	Conditions	Process Control	Interval/ Endpoint Metrics	Reproducibility
1	3	Control process scaled from client reactors	All defined process parameters met	Oxygen transfer rates higher than targeted resulting in higher productivity than observed at client site	
2	9	Control process scaled from client reactors with limit on OUR A Control process scaled from client reactors with limit on OUR B Control process scaled from client reactors with limit on OUR C The middle limit on oxygen transfer rates tested in reactors, B, resulted in performance matched to the client site and this condition was used in following experiments.	All defined process parameters met	Testing different conditions Process metrics met with one of the conditions	Data collection in progress
3	6	Control process, OUR limit B Historical process, OUR limit B	All defined process parameters met	Process metrics met	Data collection in progress
4	3	Control process, OUR limit B	All defined process parameters met	Process metrics met	Data collection in progress
Total Technical Transfer Runs	21		All defined process parameters met	Fermentation batch time +/- 1 hour of client site Final titer of strain +/- 5% of client site Final yield of strain +/- 3% of client site Maximum OD_{600} +/- 5% of client site	Within experiment CV - 3.5% Between experiment CV: 5%

Table 6: *Total runs required for technical transfer.*

CONCLUSION

Here, we described the technical transfer process for clients transferring bioprocesses to Culture Biosciences' 250mL microbial and cell culture platforms. In each case example, access to Culture's bioreactors allowed clients to perform the full set of experiments required to design and de-risk scale-up of their bioprocesses on challenging timelines. Culture's framework for successful technical transfer and downscaling, in addition to the thoroughly characterized bioreactor systems, gives clients the confidence that results generated at Culture will translate back to their facilities and beyond.

Culture is your upstream bioprocess lab, in the cloud.

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