Know, monitor and document bioproduction using Direct RNA Sequencing

Emma SALATTI, Ophélie JOUFFROY, Anne-Sophie GARY
GenSensor - France

Introduction

Bioproduction is the way to synthetise drugs from living systems (Figure 1).
Bioprocesses development and monitoring is a challenge due to inherent biological variability.

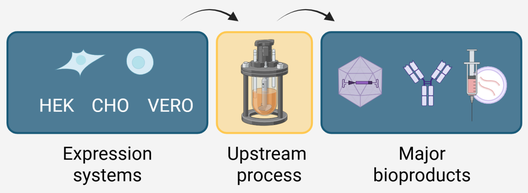
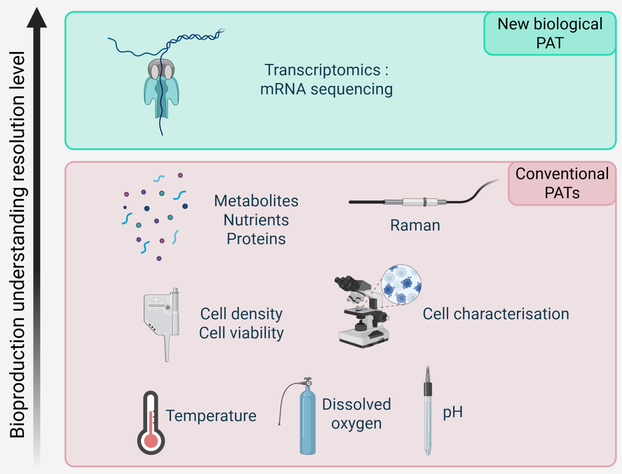


Figure 1. Illustration of bioproduction principle.



Thereby, biomanufacturing uses classic Process Analytical Technologies (PATs) to track physicochemical parameters and metabolites, providing limited insight into cellular mechanisms causing unpredictability.

Figure 2. New biological PAT increases bioprocess understanding. Conventional PATs are commonly used to monitor biologic drug synthesis.

Here, we propose an innovative biological PAT (BioPAT) that uses full-length mRNA sequencing allowing to understand cellular health, metabolic activity, and production biomarkers in near real time (Figure 2).

Methods

Suspension eukaryotic cells (HEK) were grown in flasks and transfected to produce recombinant adeno-associated virus (rAAV), with green fluorescent protein (GFP) gene as gene of interest. Cell sampling was done at several passages and timepoints, before and after transfection.

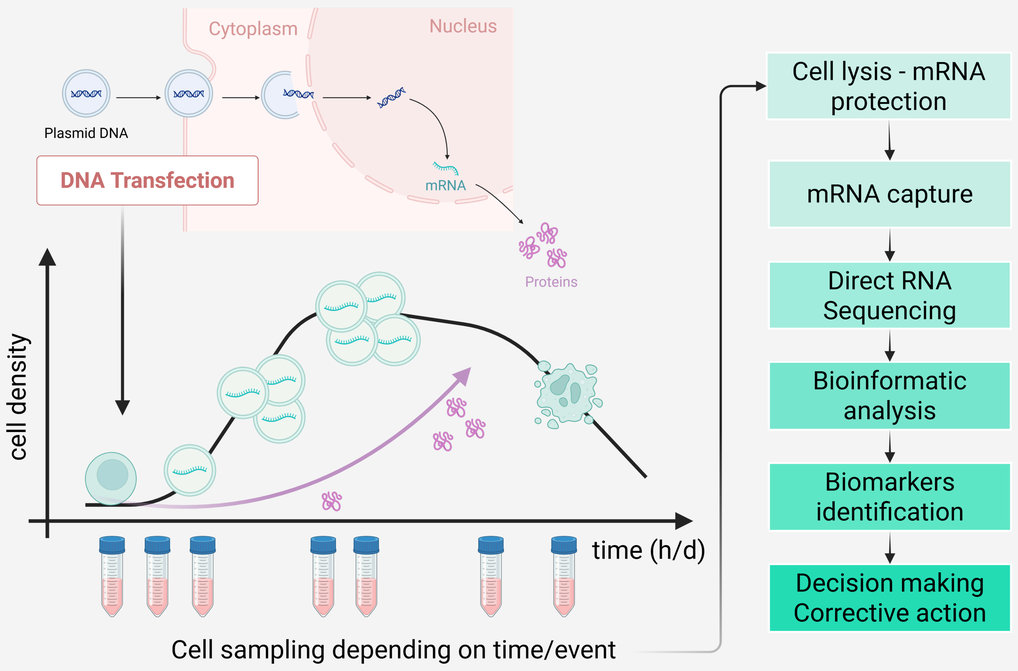


Figure 3. GenSensor's approach to monitor and comprehend bioproduction yield differences, using Direct RNA Sequencing.

After cell harvest, mRNA was extracted and prepared for direct RNA sequencing using Oxford Nanopore Direct RNA Sequeuncing kit on a GridION system. Data were analysed to define biomarkers following internal pipelines (Figure 3).

Results

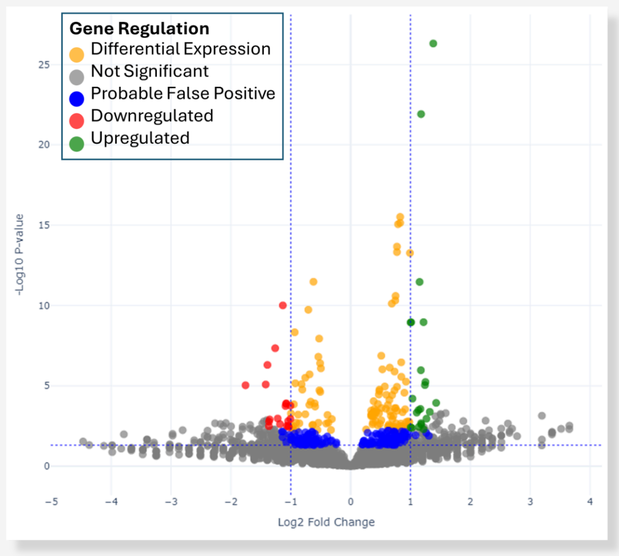


Figure 4. Differentially expressed genes for cells at two different passages before transfection (Pn+5 compared to Pn).

A yield decrease was measured between two productions.
What can explain this loss of productivity?

Conventional PATs show:
- Same cell density
- Same glucose consumption

New biological PAT measures:
- Differentially expressed genes (Figure 4)
- Strong changes in signalling pathways (Table 1)

Activated Signalling Pathway	Signficance (A.U.)	Analysis
Cytoplasmic translation	---	Translation
Mitochondrial translation	---	
Mitochondrial electron transport	---	Energy Production
Proton transmembrane transport	---	
Proton motive force-driven ATP synthesis	-	
Cellular respiration	+	
Cellular response to stress	---	Stress Response
Cell death	-	
Autophagy	-	

Table 1. Down (-) and up (+) regulated signalling pathways that explain loss of productivity between cells at two different passages before transfection (Pn+5 compared to Pn).

A decrease in yield over cell passaging due to:
- Decrease in energy production and cytoplasmic translation
- Loss of stress response, lack of plasticity
- Reduction in cells' essential activities for production

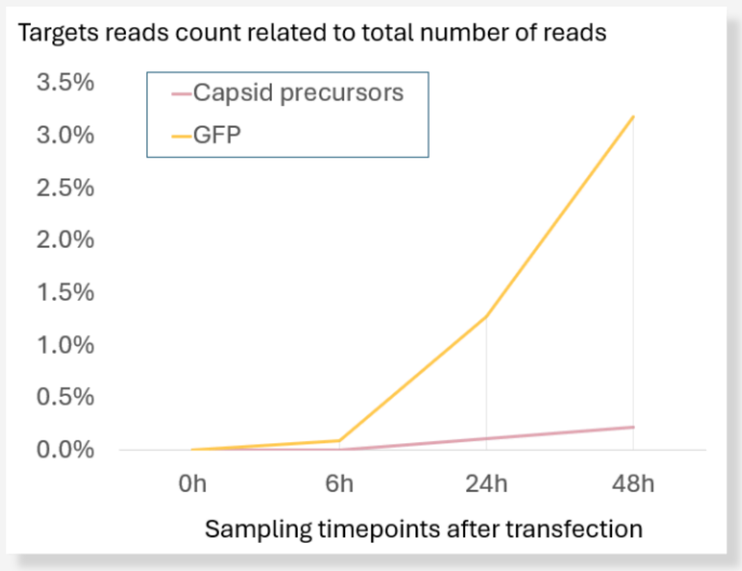


Figure 5. Kinetic tracking of capsid precursors and GFP in one sample.

Production tracking over time using a new biological PAT (Figure 5)

- GFP and capsid precursors synthesis start from 6h after transfection
- Inefficient GFP packaging

Direct RNA sequencing allows tracking of:

- ✓ Viral infection
- ✓ Transfection
- ✓ Cell culture conditions
- ✓ Cell productivity
- ✓ Environmental stimuli

Conclusion

Direct RNA Sequencing as an essential tool in bioproduction to:

- Know producing cell behaviour over time and/or through condition comparison.
- Monitor transcriptional changes during biomanufacturing to characterise cellular responses and productivity.
- Document bioprocess to optimise production yield and cell lines performance.

The new BioPAT ultimately accelerates and improves biologic production for the benefits of patients.

To know more about GenSensor's solutions and applications:
gensensor.com