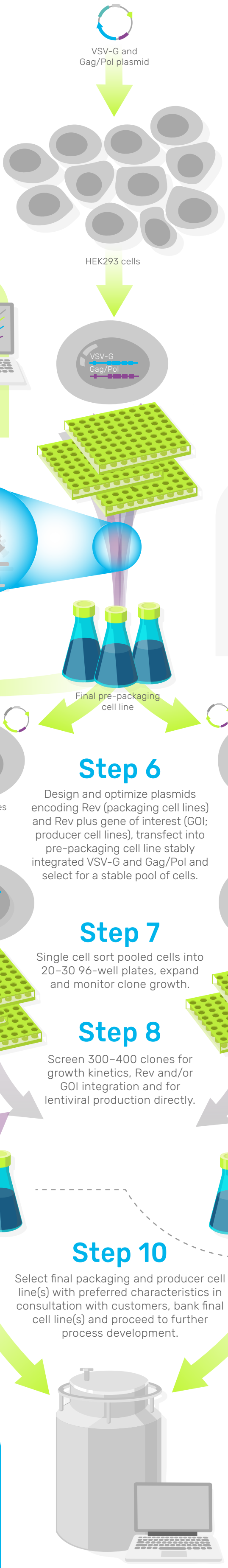
RegMedNet In focus

OXGENE™

Developing lentiviral packaging and producer cell lines



Step 1

Design and optimize the VSV-G and Gag/Pol plasmids, transfect into host cell line and select for a stable pool of cells.

Single cell sorting, clone monitoring and expansion

Automating single cell sorting, clone monitoring and expansion can enable rapid and more informed decision-making. Automated technology represents a large initial outlay; however, it could reduce process and staff costs and make your processes more efficient through higher-throughput expansion.

Step 2

Single cell sort the pool of stable cells into 50–60 96-well plates. Expand and monitor clone growth.

Step 3

Screen clones for growth kinetics, VSV-G and Gag/Pol integration, and expression with and without induction. Screen 500–600 clones for transgene presence, 40–50 clones for transgene expression and 10 clones for lentiviral production.

Step 4

Further expand, characterize and optimize growth and lentiviral production conditions for top performing clones including tests to confirm growth profile, VSV-G and Gag/Pol copy number, optimal culture media, cell density at transfection, transfection reagent and supplementation.

Step 5

Select the final pre-packaging cell line for banking, test for stability and further rounds of cell line development to establish packaging and producer cell lines.

Step 6

Design and optimize plasmids encoding Rev (packaging cell lines) and Rev plus gene of interest (GOI; producer cell lines), transfect into pre-packaging cell line stably integrated VSV-G and Gag/Pol and select for a stable pool of cells.

Step 7

Single cell sort pooled cells into 20–30 96-well plates, expand and monitor clone growth.

An alternative route to develop producer cell lines from lentiviral packaging cell lines

Step 8

Screen 300–400 clones for growth kinetics, Rev and/or GOI integration and for lentiviral production directly.

Step 9

Further expand, characterize and optimize growth and transfection/induction conditions for top performing clones including tests to confirm growth profile, VSV-G, Gag/Pol, Rev and/or GOI copy number, cell density at transfection/induction and supplementation.

Step 10

Select final packaging and producer cell line(s) with preferred characteristics in consultation with customers, bank final cell line(s) and proceed to further process development.

Step 11

Carry out further process development to optimize growth conditions (both packaging and producer cell lines), transfection conditions (packaging cell lines) and improve final titer. The transfection-free nature of producer cell lines makes them suitable for more extensive process development than cell lines that must undergo transfection.

This infographic was created as part of the RegMedNet In Focus on packaging and producer cell lines in association with OXGENE

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