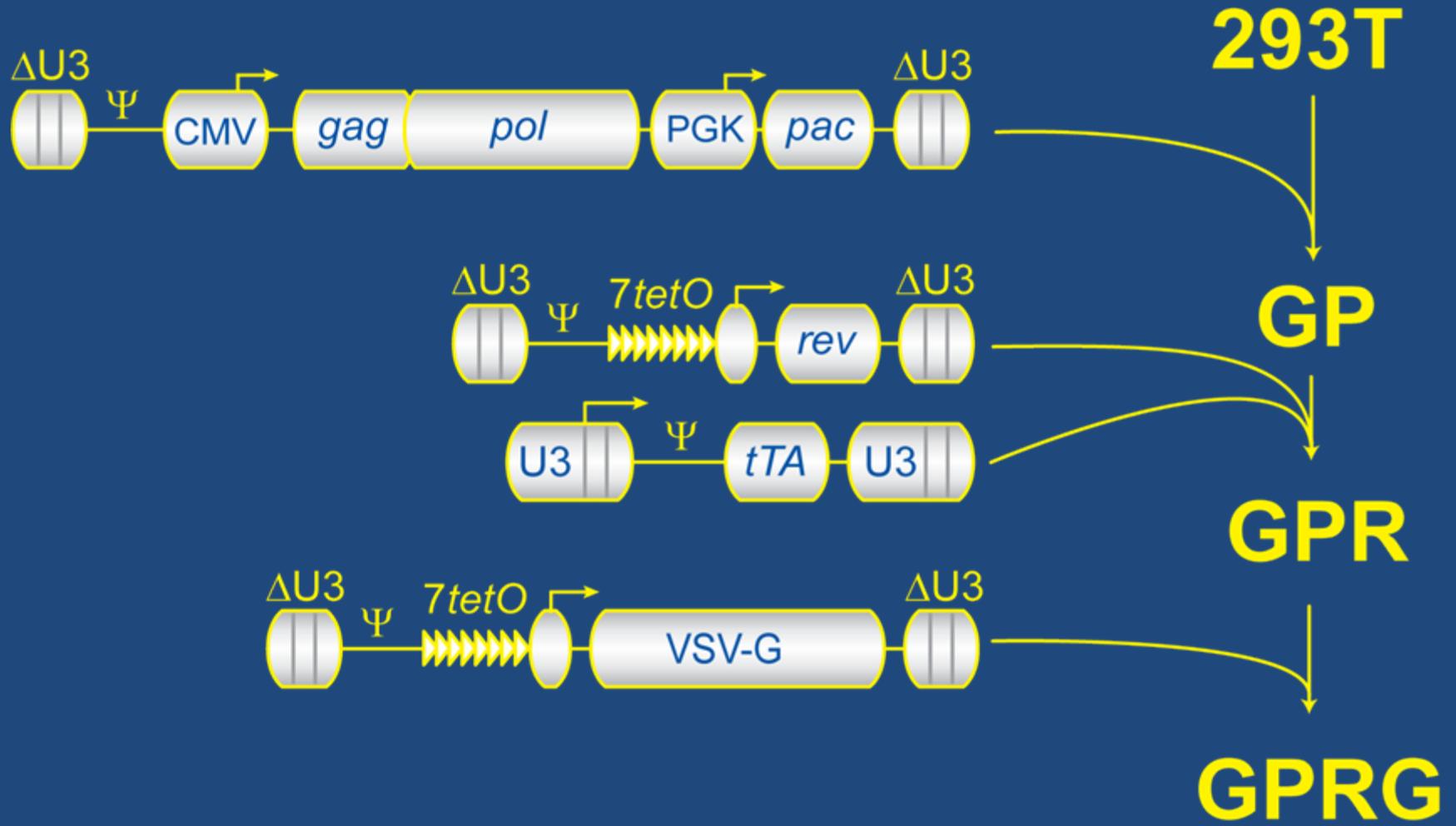


# Stable Lentiviral Vector Production System

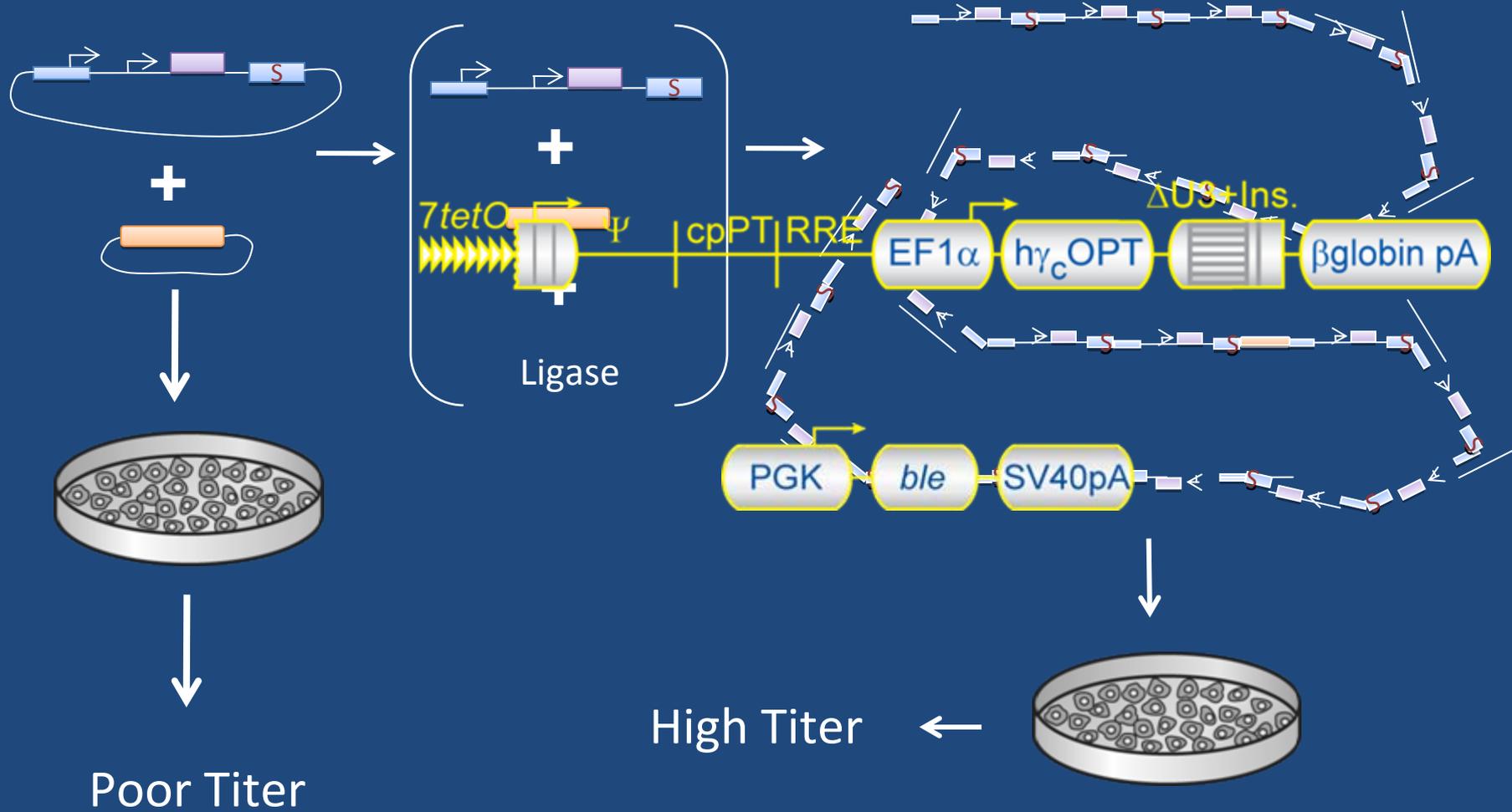
John T. Gray, Ph.D.

Director, Vector Production and Development  
St. Jude Children's Research Hospital

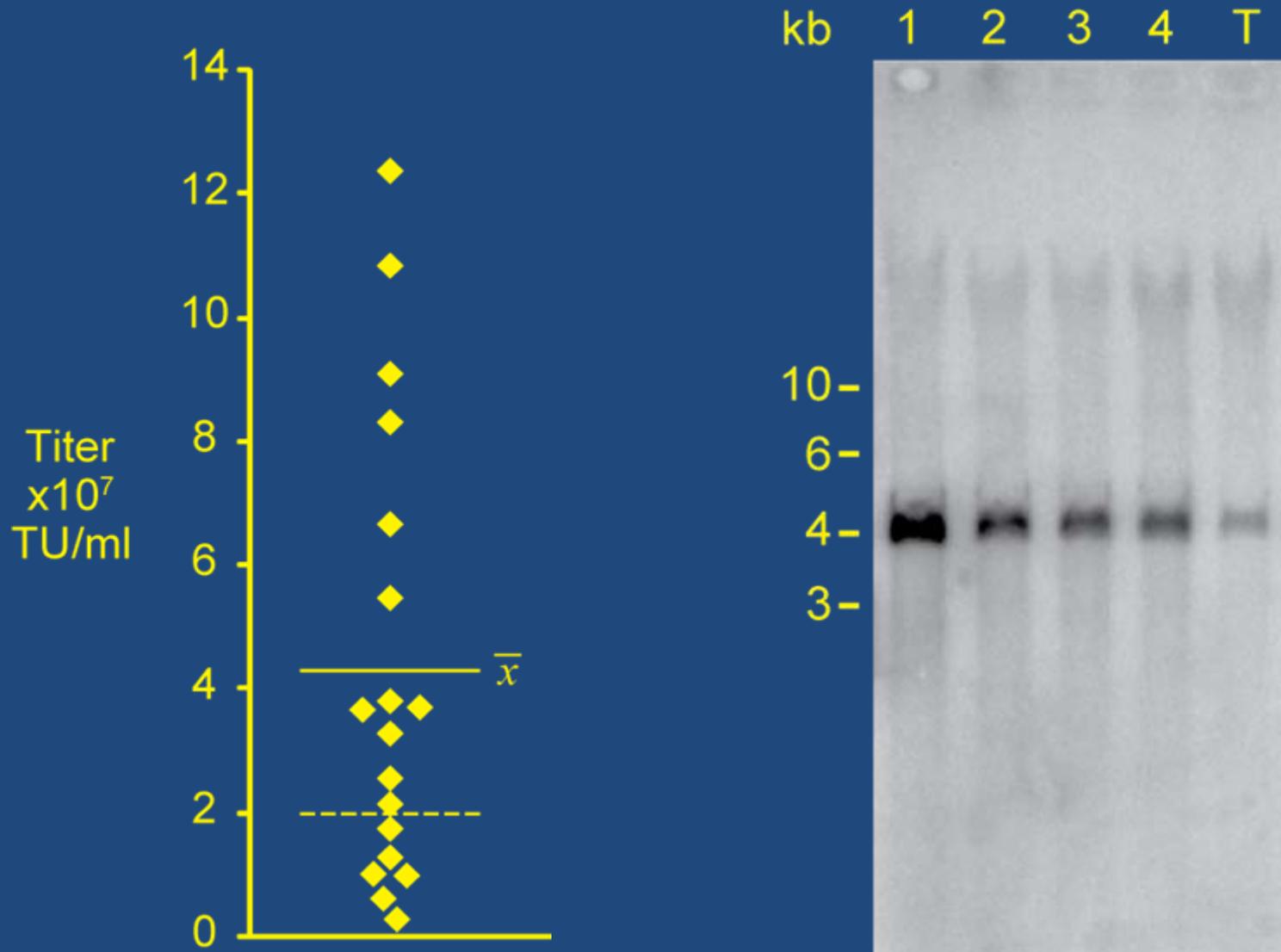
# Clinical Lentiviral Vector Packaging Lines



# Concatemeric Array Transfection



# SCID-X1 Vector Producer Clones



# Clinical Vector Production Process

MCB



Expand cells

- 1 nM doxycycline
- No other antibiotics
- 2-4 weeks



WAVE Cell Bag Culture

- Fibracel Disks for cell attachment
- 7 Days expansion with 1 nM doxycycline
- Wash cells to remove doxycycline and induce
- Harvest vector daily
- Optionally concentrate with tangential flow filtration



# Functional Product Validation

- All product is titrated on ED7R cells, a human T-cell line with little to no  $\gamma$ -chain expression
  - XVIVO-10 (serum free) harvest:
    - $1-3 \times 10^7$  tu/ml unconcentrated
    - $1-3 \times 10^8$  tu/ml after concentration by TFF
  - DMEM+10% serum harvest:
    - $4-6 \times 10^7$  tu/ml
  - Continuous production for 5-8 days
- Transduction of Human CD34+ Cells

Source of Cells	Vector Product	Conditions	CFU Transduction
Healthy volunteer steady state bone marrow	From research cell bank, unconcentrated, harvested in DMEM + 10% serum	MOI = 20, two hits, with serum	22/32 (69%) colonies positive by PCR
Healthy volunteer steady state bone marrow	From research cell bank, unconcentrated, harvested in DMEM + 10% serum	MOI = 25, two hits, with and without serum	No serum: 14/20 (70%) Plus serum: 13/20 (65%)
Healthy volunteer steady state bone marrow	From Master Cell Bank grown in bioreactor, unconcentrated, harvested in DMEM + 10% serum	MOI = 25, two hits, with or without prestim.	No prestim: 14/24 (58%) Plus prestim: 15/24 (63%)

# Summary

- Concatemeric Array Transfection enables efficient derivation of stable producer clones for self-inactivating vector genomes
- Stable production system capable of producing up to 150 liters per run allowing treatment of all patients from both trials with a single, uniform batch of vector product
- Product generated from GMP grade Master Cell Bank, using GMP-compatible process, efficiently transduces human CD34+ cells
- Eliminates the use of plasmid DNA in production process, which enhances safety and product uniformity
- Producer line has been tested for replication competent lentivirus and inadvertent transmission of helper components (both not detected)