Stanford MEDICINE

Academic Cell and Gene Therapy Development and Manufacturing

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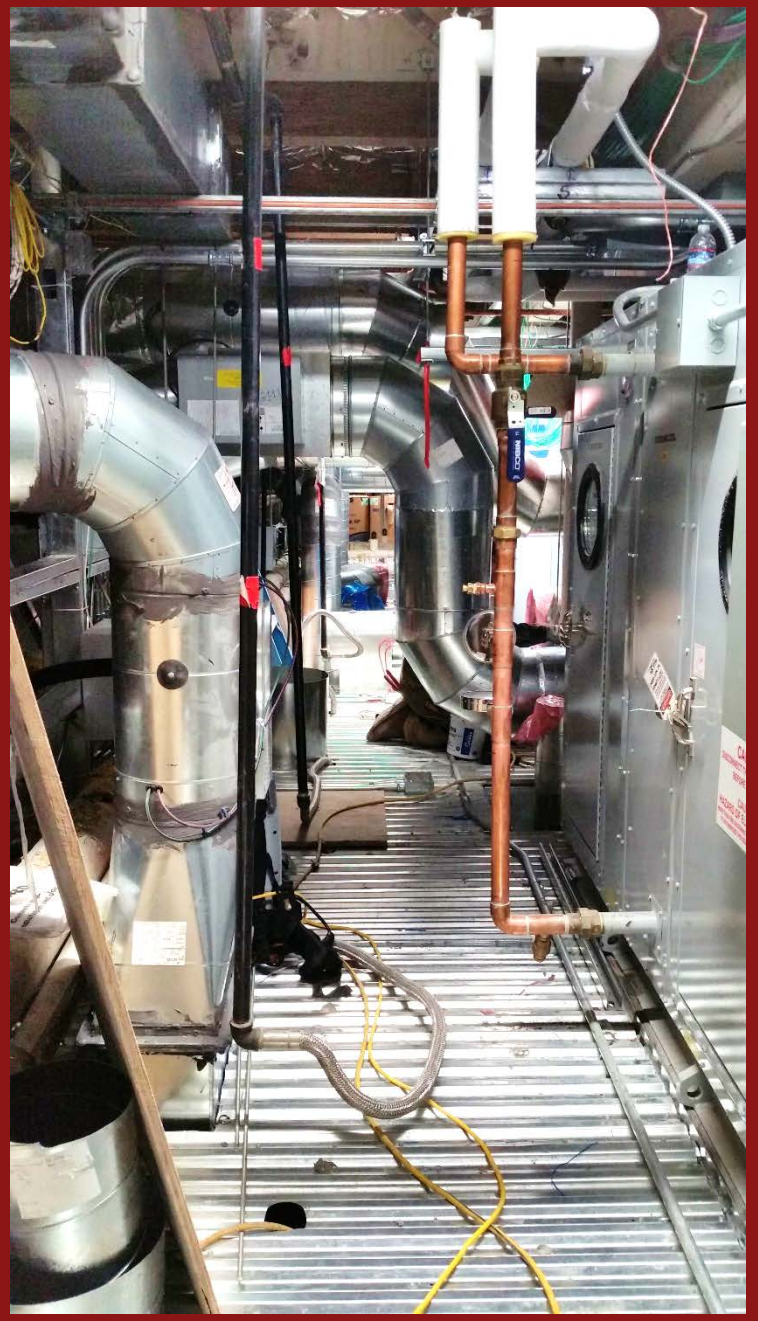
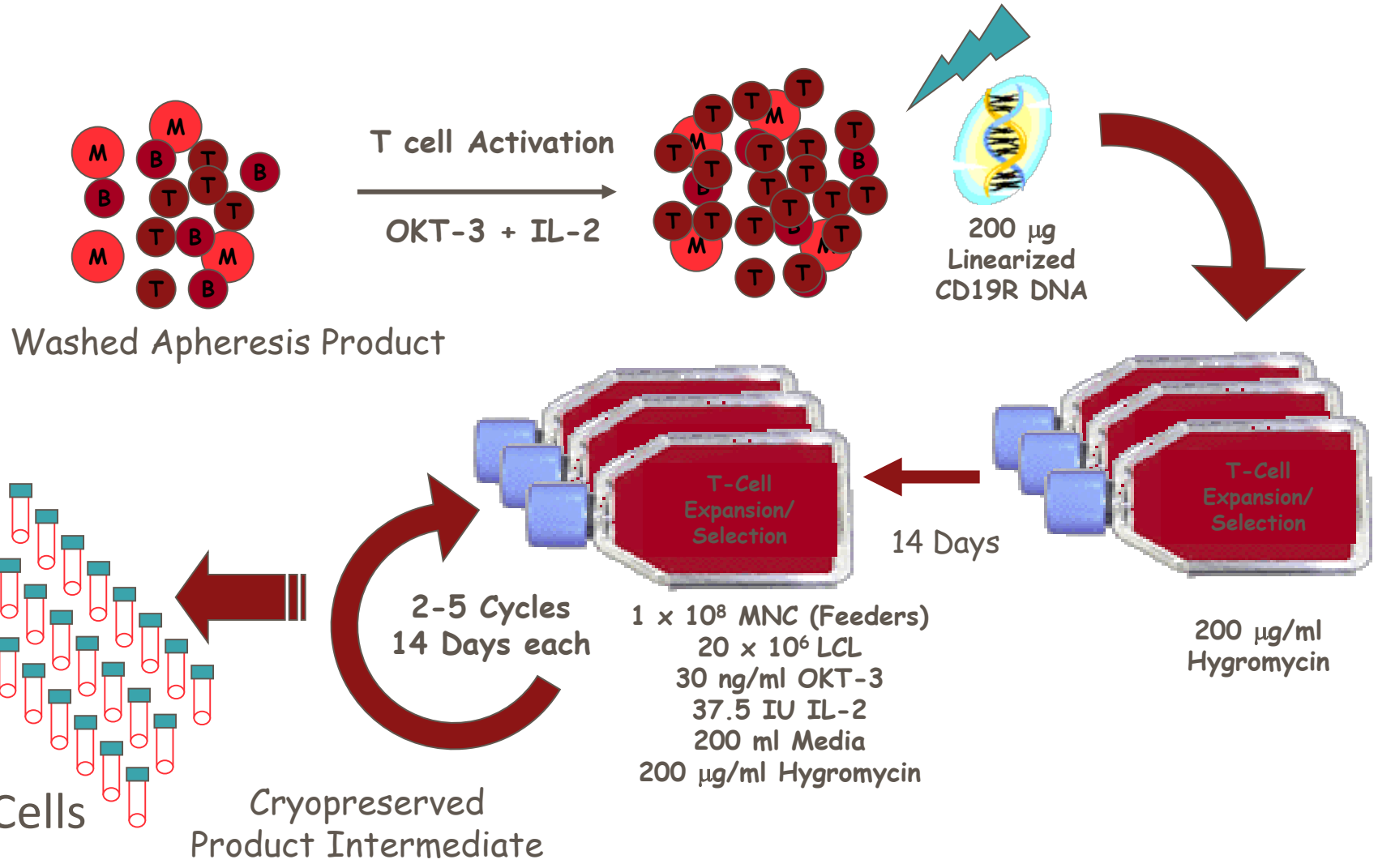


T-cell Manufacturing Process circa 2000



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Fast forward 2016 - Bi-Specific CAR-T cells

- Process Challenges
  - Closed system processing of Apheresis products
  - CD4/CD8 T-cell enrichment required
  - Lentiviral Transduction in closed system
  - Bioreactor culture and harvest in closed system
- Analytical Challenges
  - Demonstrating simultaneous expression of both CARs
  - Deciding on in process metrics
  - Rapid reliable assay for RCL detection

Closed and (Semi) Automated Blood Cell Culture Systems

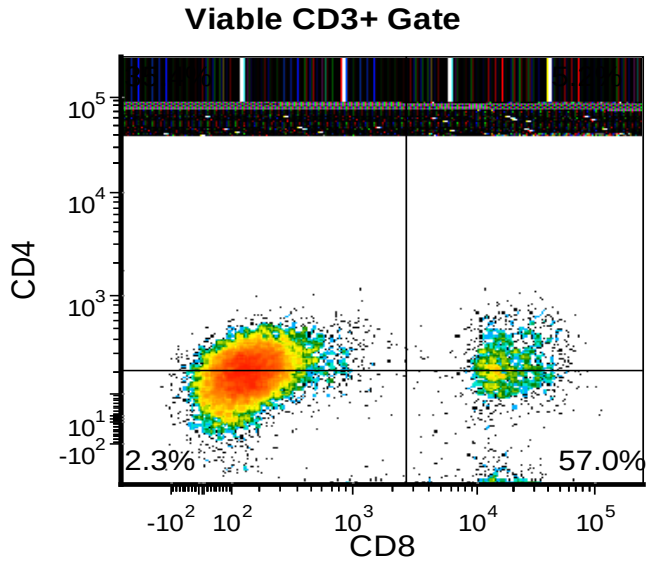
7 Day Process on Prodigy

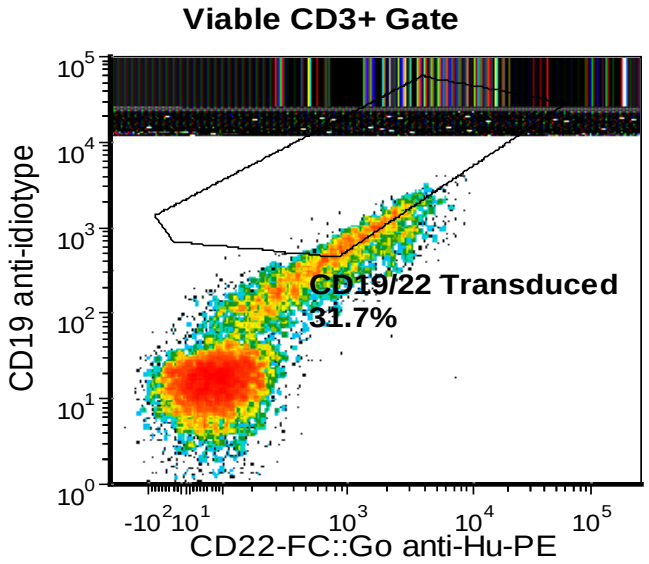
- Apheresis Washing
- Magnetic Bead Labelling
- Target Cell Enrichment
- Cell Growth Formulation
- Cell Stimulation
- Viral Transduction
- Cell Expansion
- Cell Harvest
- Release Testing

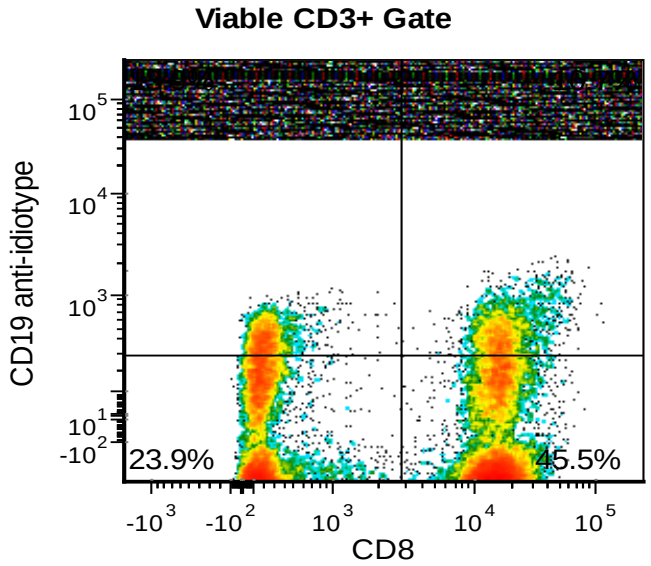
CliniMACS Prodigy

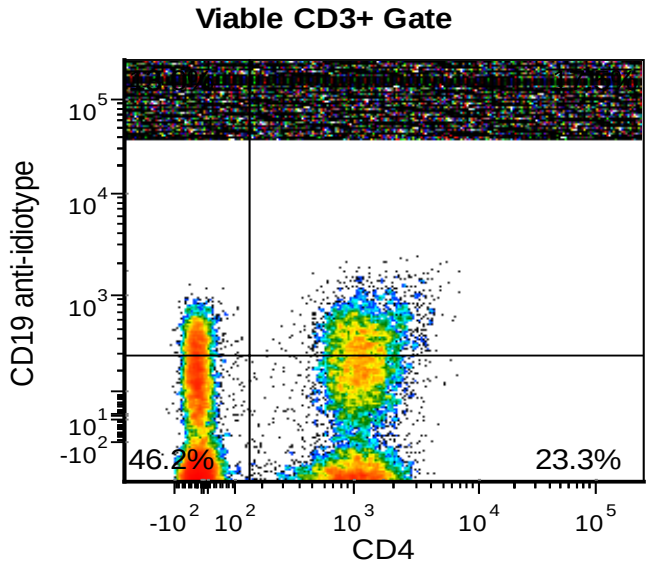
CAR T-cells

Phenotypic Analysis of CD19/CD22 bi-Specific CAR-T





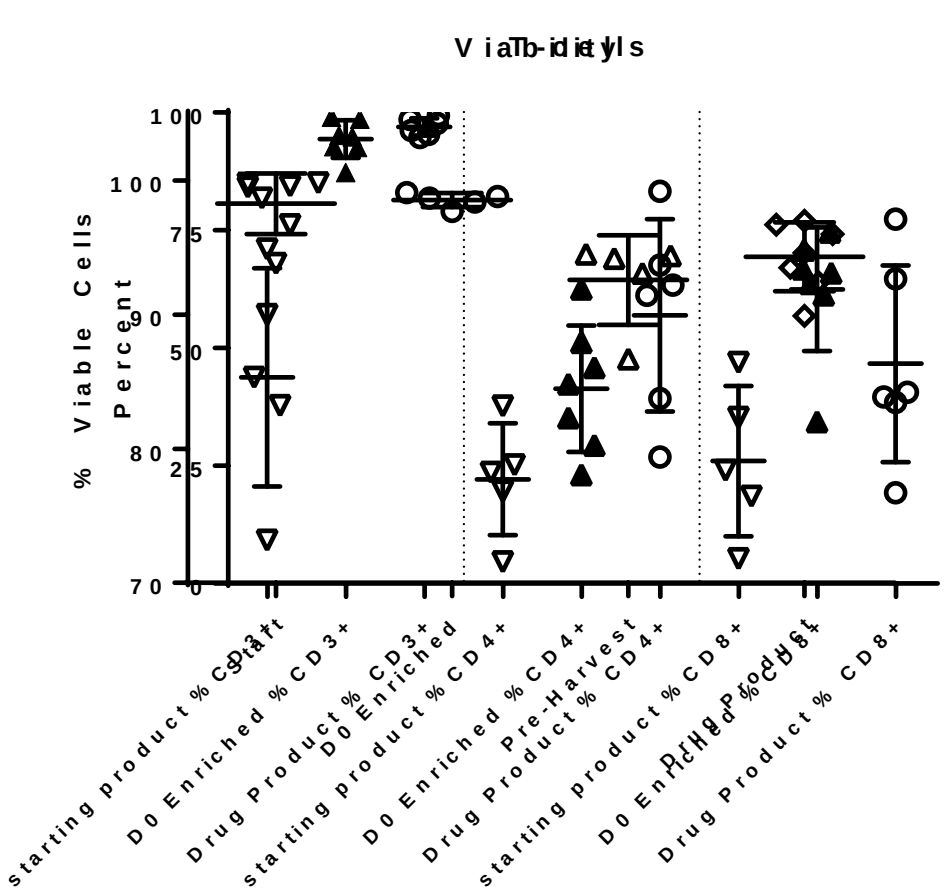


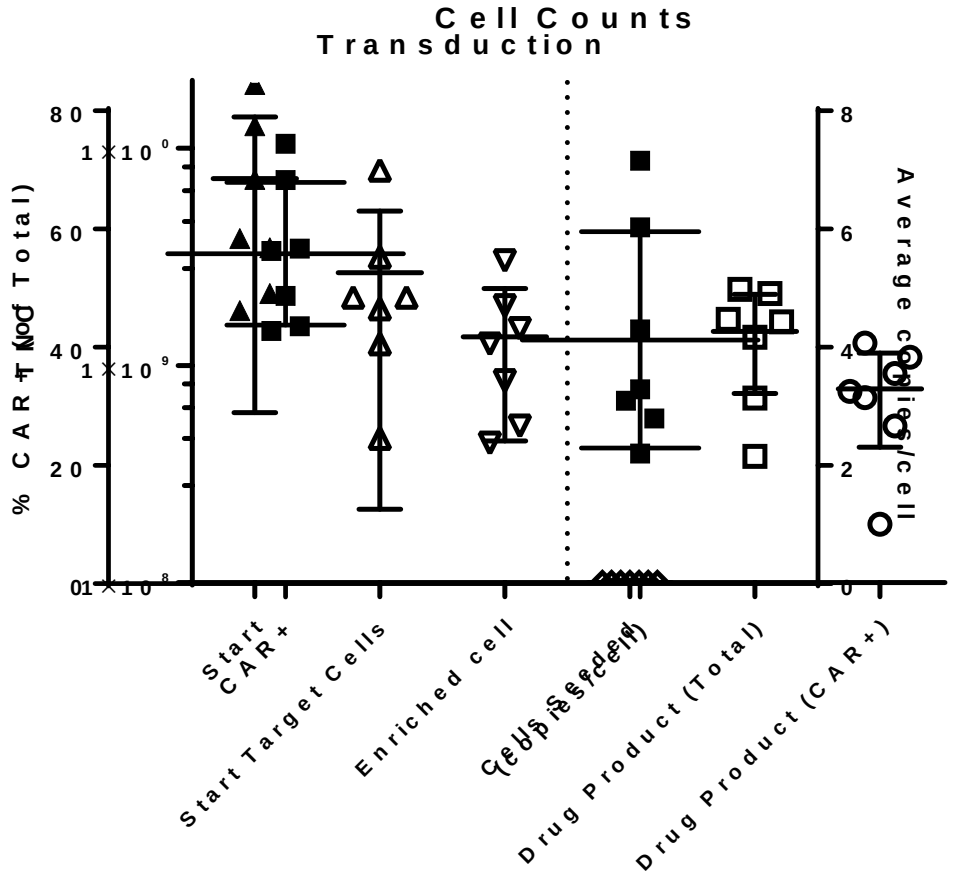


Anti-CD19 idotypic antibody to CD19 CAR (Laurence Cooper)

rhSiglec 2/Fc Chimera
(CD22 on Ig stalk – R&D Systems)

CAR-T Production Metrics





Production summary

- Pros
  - Reproducible production of CAR-T cells
  - All cells passed all release testing
  - Semi-Closed system for production
    - Still open harvest/formulation steps performed in BSC

- Cons
  - Sole source provider for all reagents/tubing sets
  - Multiple device failures (valves, motors, tubing sets)
  - Expensive incubator for 6/7 days
    - Limits productivity in GMP suite

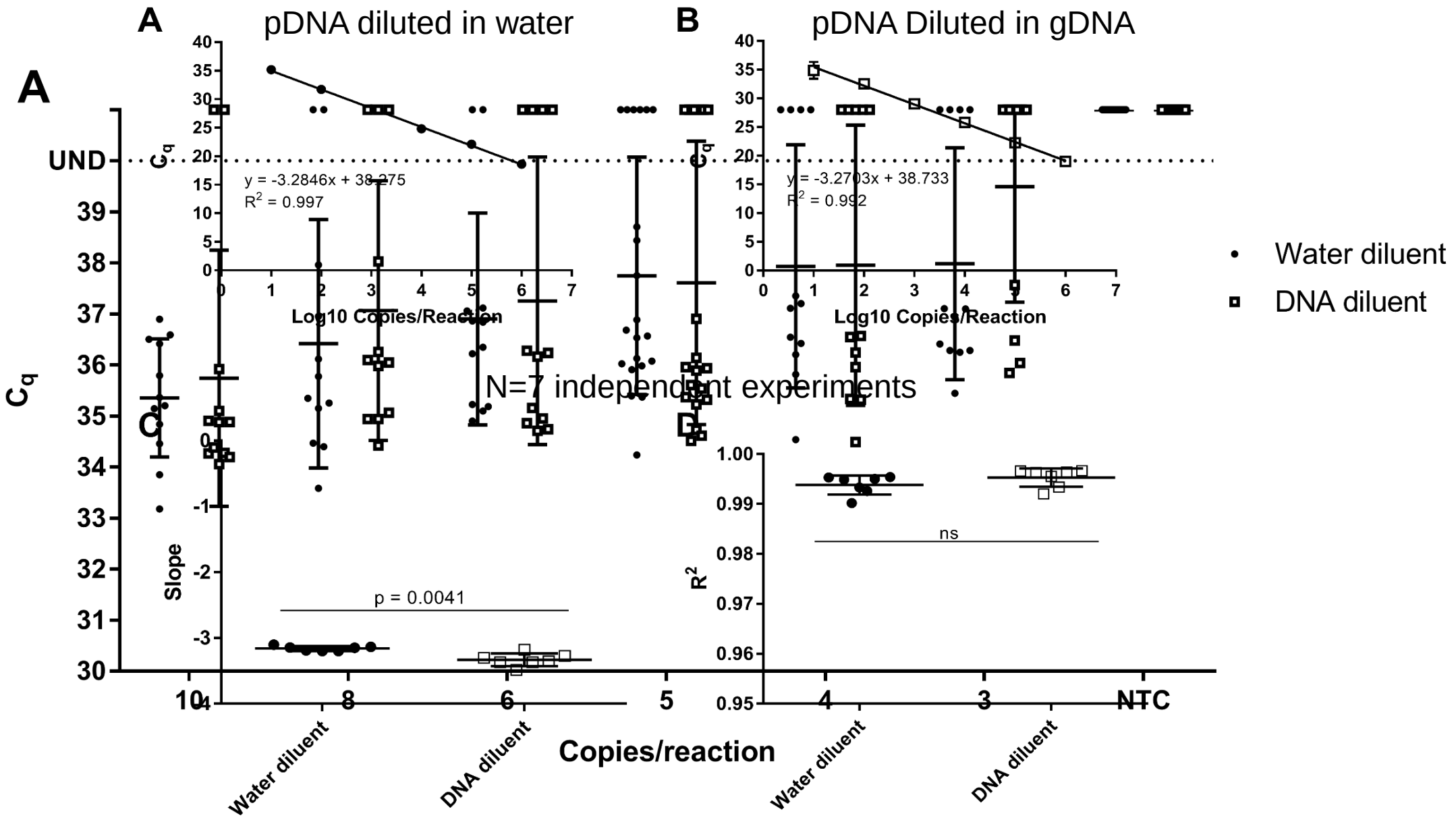
qPCR for detection of RCL

- Using VSV-G as a target
  - All lentiviral constructs contain VSV-G envelop sequences
  - Env is on a helper plasmid and should not be contained in transduced T-cells
  - RCL could be formed in VSV-G env is transferred to T-cells

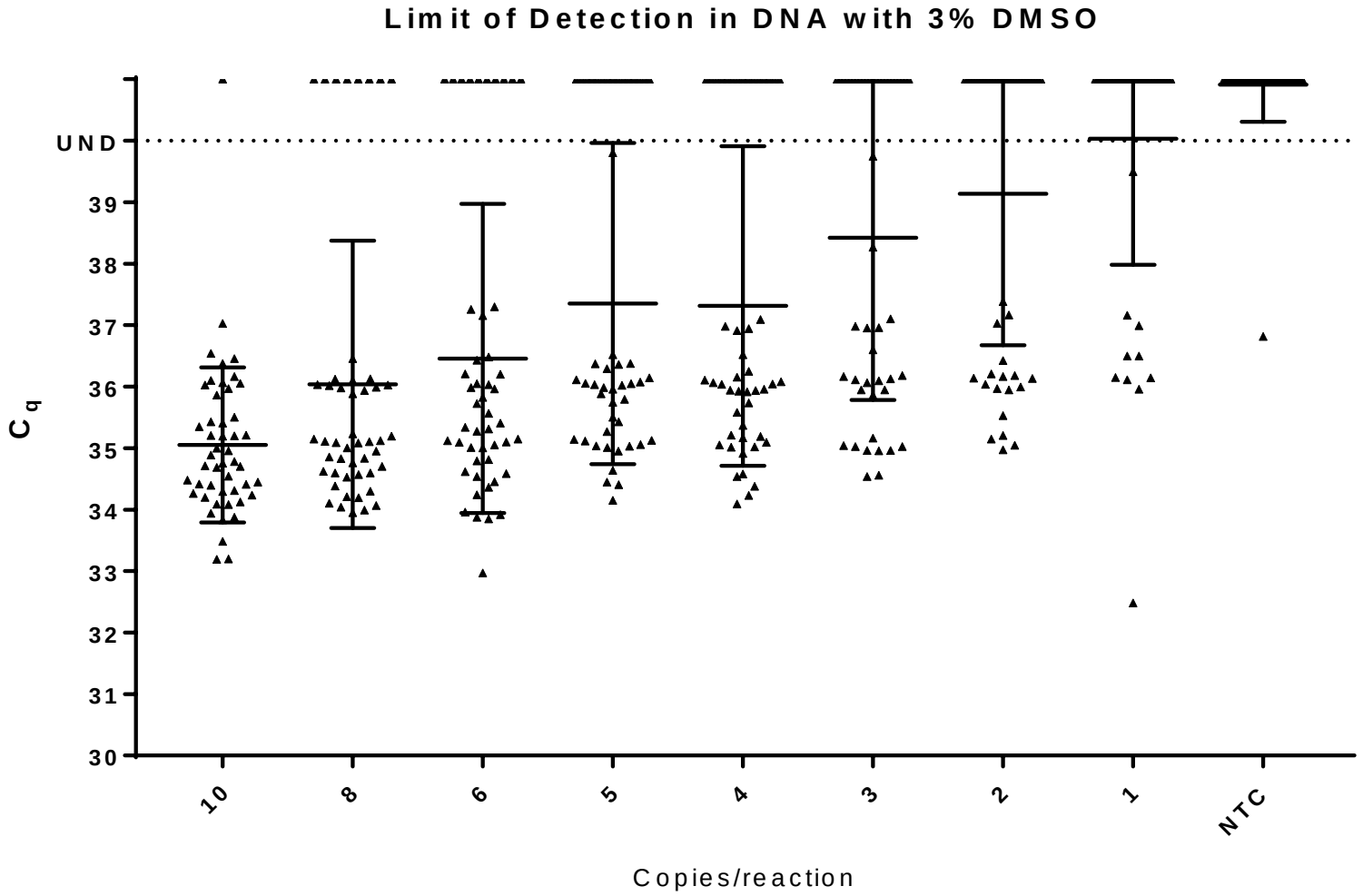
- Determine Quality of the assay (MIQE)
  - Carefully quantified plasmid DNA as Control
  - Linearity, slope are as predicted by dilutions
  - Specificity and Limit of detection is established
    - Measure frequency of false positive and false negative

Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE)

Linearity and slope of qPCR of VSV-G Plasmid



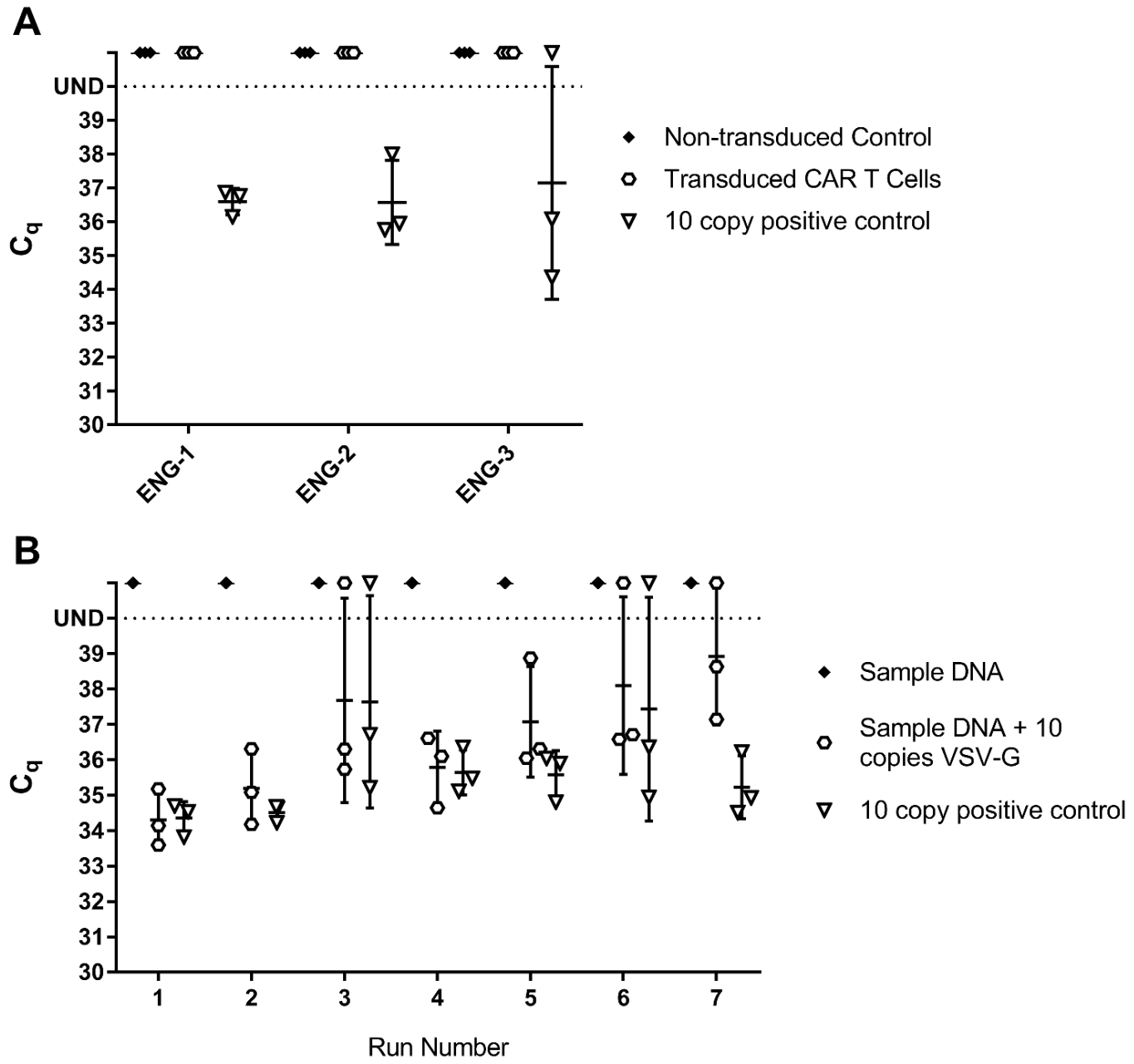
Inclusion of 3% DMSO Improves Detection of Low Copy Number DNA



	Water Diluent	DNA diluent	DNA diluent + 3% DMSO
10 copies	100.00%	83.33%	97.92%
8 copies	83.33%	75.00%	83.33%
6 copies	83.33%	66.67%	79.17%
5 copies	76.19%	61.90%	68.75%
4 copies	66.67%	58.33%	68.75%
3 copies	66.67%	33.33%	52.08%
NTC	0.00%	0.00%	2.08%

N = 16 independent experiments in triplicate

Pre-Clinical Qualification Runs and First 7 patient samples



Analytical Summary

- Pros
  - Simultaneous Detection of expression of both CAR proteins
  - Reproducible, sensitive RCL assay
    - Reliable detection of 10 copies of VSV-G
    - False Negative rate <3%
    - False Positive rate ~2%
    - Low Inter-operator variability
  - All testing completed and reviewed on day of product harvest (12 hours)

- Cons
  - Requires clean, dedicated space for assays
  - Requires carefully quantified source of control DNA
  - Approximately 1/40 products may be falsely considered positive for RCL
  - Approximately 1/33 products may miss 10 copies of RCL

Acknowledgments

- LCGM CAR-T Production Staff
  - Lindsey Skrdlant, Kerri Tate, Cindy Tudesco, Brian Fox
- LCGM Analytical Team
  - Brett Keidaisch, Mario Lorente, Randall Armstrong
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  - Stanford Cancer Immunology and Immunotherapy Program (**Crystal Mackall**)
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  - NCI – Terri Fry

Thank You for Your Attention!