Approaches to Potency Assays of Pre- and Prepro-Biologic Vaccines:

The role of RT-PCR and Genetic Stability in characterizing potency of plasmid DNA-based vaccines

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Outline

- Definition & Context
  - Pre- & Prepro-Biologics: pDNA Vaccines
  - Potency v Strength
  - Conventional v Non-conventional
  - Key Assumptions
- Potency Assays of Pre- & Prepro-Biologics:
  - Genetic Stability
  - Potency: the immediate “given result”
  - RT-PCR
  - In vitro – in vivo correlate
- Summary

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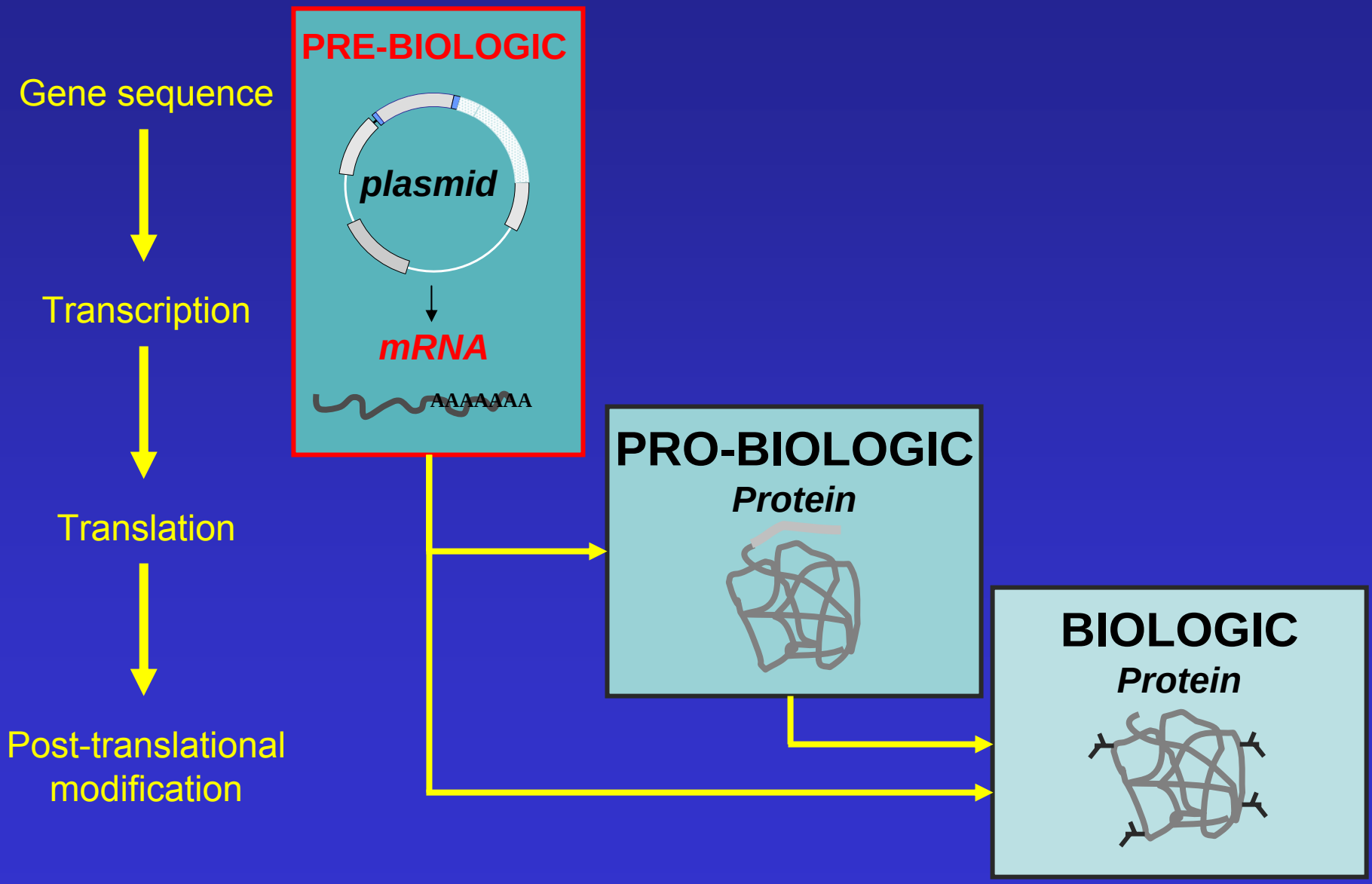
Definition: Pre- & Prepro-Biologics



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Evolution in approach to vaccine regulation: Two paradigms

- "OLD: Vaccine potency, as measured in the laboratory, is the most important characteristic to ensure human efficacy"

- "NEW: Vaccine potency is only one of the tools used to ensure that a manufacturing process yields immunobiologicals of quality consistent with that of lots proven efficacious"

From: **Assays and laboratory markers of immunological importance**

Bruce D. Meade & Juan L. Arciniega
Laboratory of Methods Development and Quality Control
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February 2001

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Context: Potency v Strength

"Potency of a vaccine is a little bit like the strength of a drug, but more complex."

From:

Glossary. Vaccine Pre-clinical Toxicology Testing by P.Y. Chang, Ph.D., CDR Rebecca Sheets, Ph.D., Stuart Shapiro, M.D., Ph.D., Sally Hargus, Ph.D., and Marion Gruber, Ph.D.

http://www.niaid.nih.gov/daids/vaccine/Science/VRTT/11_Glossary.htm

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Context: Vaccine Potency

Vaccine Type	Strength	Potency
Conventional		
Live-attenuated	PFU, $TCID_{50,}$ MQPA	PFU, $TCID_{50,}$ MQPA
Killed, whole	Immunoassay of Ag	Mouse ED_{50}
Subunit		
Protein	mcg	Mouse potency, IVRP
Carbohydrate	mcg	Rate Nephelometry
Non-conventional		
pDNA	**???**	**???**

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Key Assumption
Strength

- Dose (*Strength*) of pDNA vaccines based on DNA concentration:
  - A_{260} (or equivalent)

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Key assumptions-Part 1 of 3
Potency

- Laboratory animal immune responses to pre- & prepro-biologic vaccines are not highly predictive of immune responses in humans
- *In vivo* assays have high inherent variability

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Key Assumptions-Part 2 of 3
Potency

- If the immediate biological activity of a pre-pro-biologic vaccine is to ***effect*** transcription of an immunogen, then the immediate biologic ***result*** of the product is mRNA.

Context: Vaccine Potency

Vaccine Type	Strength	Potency
Conventional		
Live-attenuated	PFU, $TCID_{50,}$ MQPA	PFU, $TCID_{50,}$ MQPA
Killed, whole	Immunoassay of Ag	Mouse ED_{50}
Subunit		
Protein	mcg	Mouse potency, IVRP
Carbo	mcg	Immunoreactivity
Non-conventional		
pDNA	**A_{260}**	**Immediate given result = mRNA**

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Key Assumptions- Part 3 of 3

- If a Pre-biologic vaccine is genetically stable, then:
  - there will be no lot-to-lot variability of primary **nucleotide** sequence
  - there will be no lot-to-lot variability of primary, secondary or tertiary **protein** structure
  - the only *potential* lot-to-lot variability of the drug substance is strength and higher order DNA structure

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Genetic Stability
What & How

- Characterization (not release) assay
- Determined once for a MCB/WCB
- Stepwise approach completed as part of commercial-scale process validation
  - At IND:
    - Sequence of MCB/WCB
    - Restriction fragment size pattern on drug substance
  - During clinical development:
    - Intermediate analysis to identify risk
  - By commercial filing:
    - Complete analysis of plasmid backbone at full-scale
    - Statistically significant GXP analysis of expression cassette at full-scale

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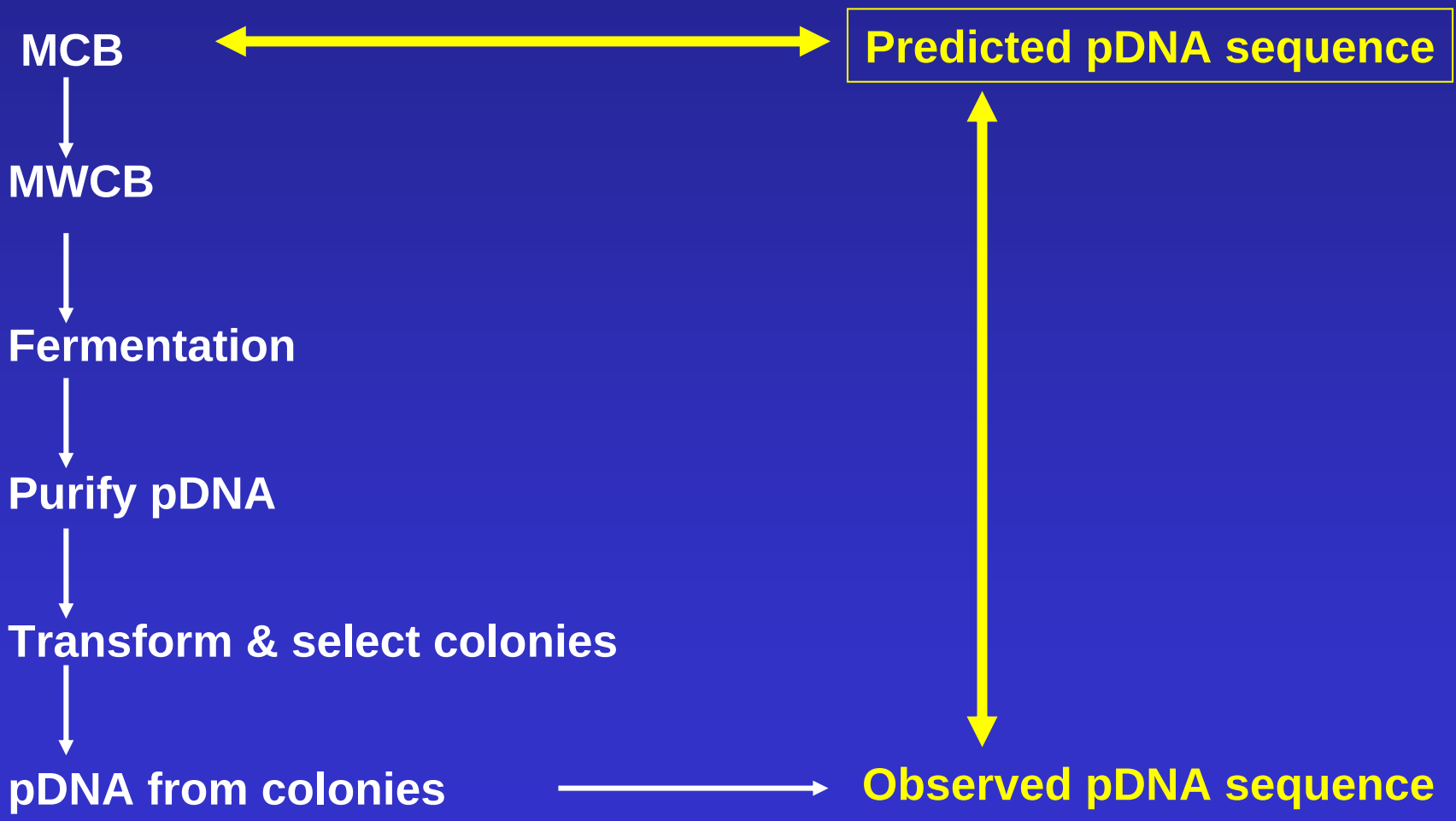
Genetic Stability

Protocol Overview



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Genetic Stability
Protocol Overview

- Mimic full-scale fermentation from MWCB (meet or exceed the number of generations in typical full-scale production fermentation)
- Isolate pDNA from fermentation broth and transform competent bacteria
- Select statistically appropriate number of “re-transformed” clones
- Grow-up and independently isolate pDNA
- Sequence with sufficient redundancy
- Compare to the MCB sequence & predicted sequence

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Genetic Stability

Example of Sample Size and Confidence Level

Sample Size	Mutants	Probability
20	0	12.16%
30	0	4.24%
50	0	1.70%
60	0	0.90%

There is a >95% probability of detecting one or more mutations in a sample of 30 independent clones if the actual mutation prevalence is >10%.

A >99% probability of detection for a mutation prevalence of >1% would require 459 independent clones

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Genetic Stability

Impact on Release Assays

- Need for any immunoblot analysis?
- Need for potency assay on drug substance, if strength (nucleic acid concentration) and structural analysis (e.g., % circular v linear) specifications are set?

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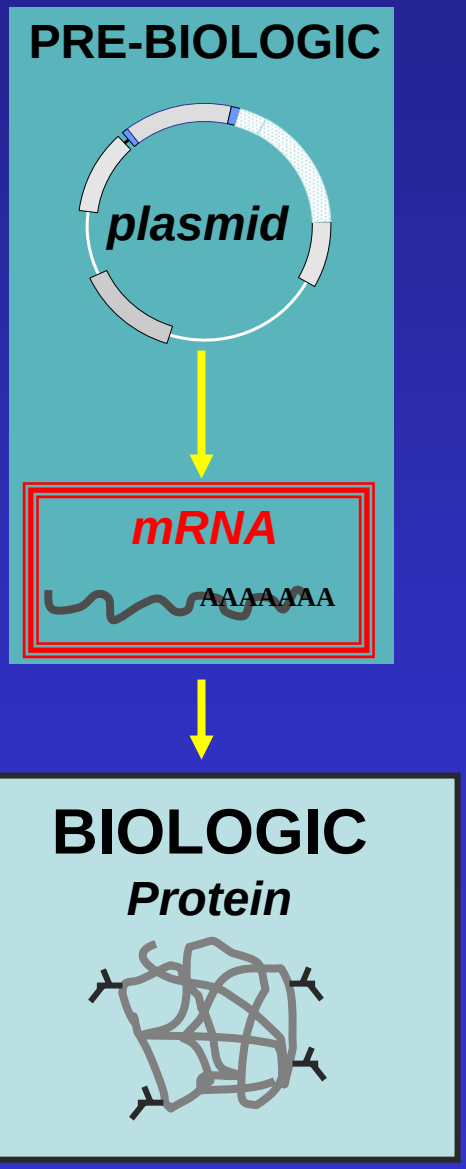
Pre-Biologic Vaccines:
Immediate given result

Gene sequence

Transcription

Translation

Post-translational modification



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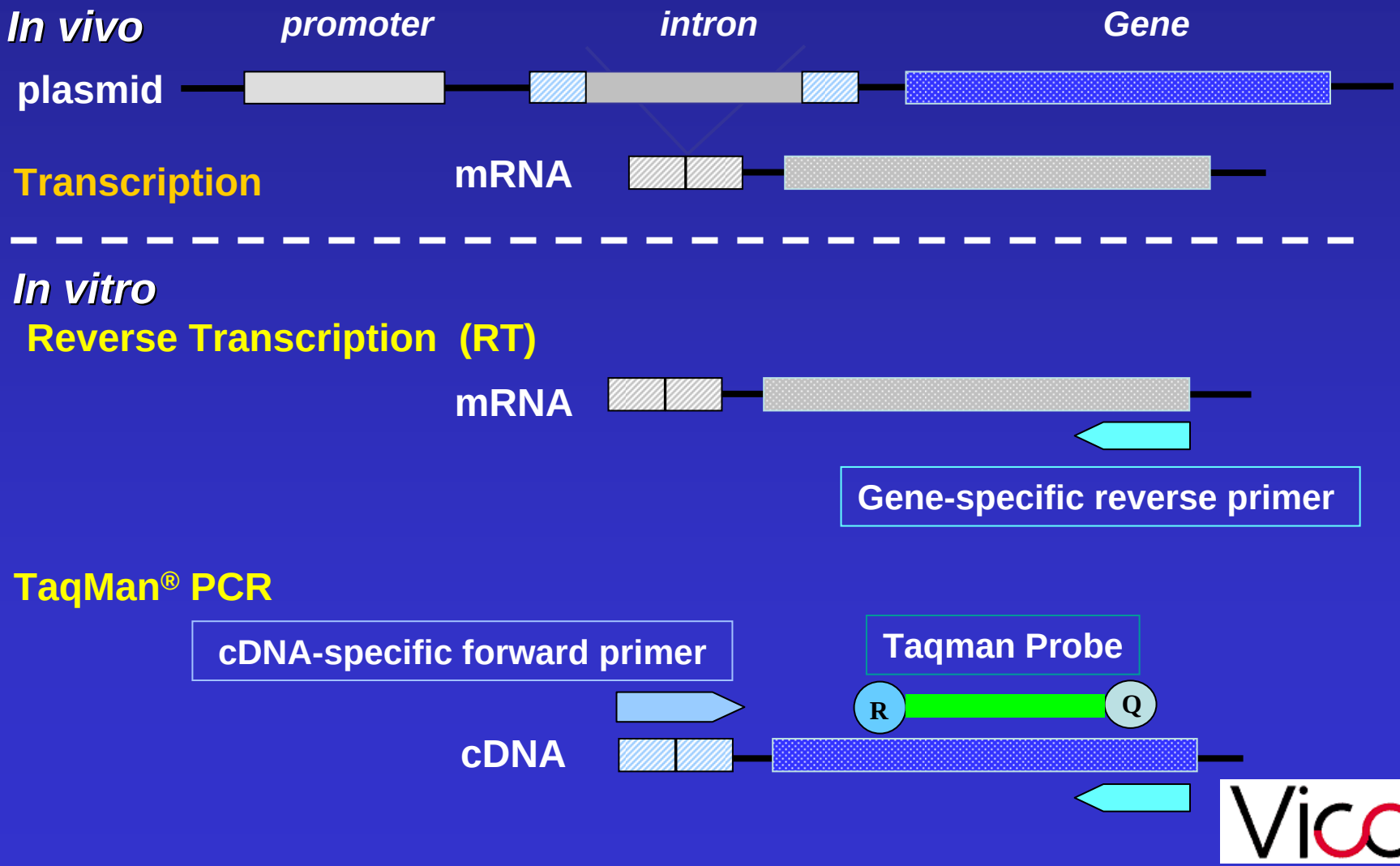
Potency assay

RT-PCR & Specificity of PCR primer



Potency assay
RT-PCR %RP Assay Rationale

Drug Product

- Pre-Biologic
- Prepro-Biologic

RT-PCR Assay

Attributes

- Measures the immediate biological effect (transcription)
- "Quantitative"
  - Large dynamic range
  - Low inherent variability
- "Specific"
- "Validatable"
- Reagents: invariable, stable, readily available

PROTEIN

FACS, ELISA Assay

Attributes

- Measures distant biological effect (cell substrate dependent)
- "Quantitative"
  - Narrow dynamic range
  - High inherent variability
- "Specific"
- "Validatable" (w/ difficulty)
- Reagents: lot-to-lot variability, unstable, long development time

Potency assay

Case Study

Development of CMV Vaccine
RT-PCR Percent Relative Potency (%RP)
Assay

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Potency assay
Poloxamer-formulated pDNA-based vaccine

- DNA vaccine
  - hCMV gB
  - hCMV pp65

  } ***Bivalent***
- Plasmid backbone
  - Proprietary Vical design
  - Tested in prior clinical trials
- Formulation
  - 2 pDNAs (5mg/mL)
  - CRL1005 poloxamer (7.5mg/mL)
  - BAK (0.11 mg/mL)
  - PBS

Vaccine to protect against CMV-associated disease

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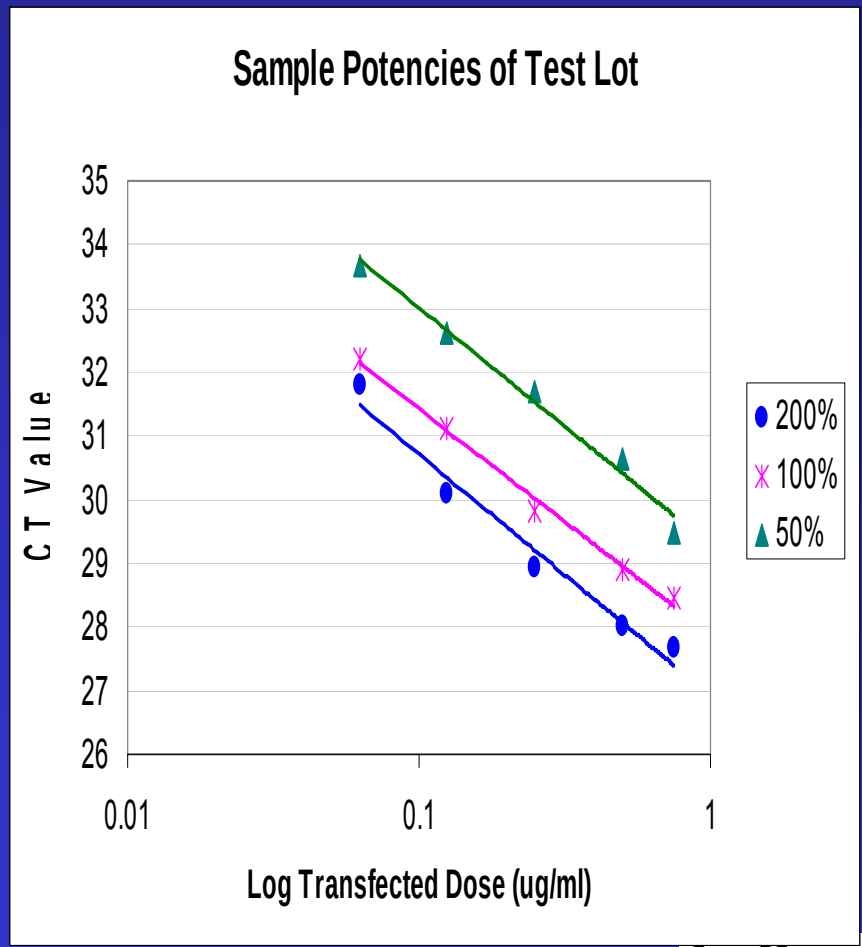
Potency assay

Test Potency Samples

- Evaluate sample potencies
  - Prepare samples of different potencies based on concentration
- Examined potency ranges
  - 50% to 200%
- Observation & model
  - Dose range plots for various potencies conform to parallel line model

Expected Potency	Observed Potency
50%	54%
75%	88%
150%	135%
200%	194%



Potency assay

Qualification Results and Proposed Validation Criteria

	gB (VCL-6365)	pp65 (VCL-6368)
Dose response model fit	Parallel line	Parallel line
Dose treatment (x-axis)	Log-transformed	Log-transformed
4-dose set (µg/mL pDNA)	0.0625, 0.125, 0.25, 0.75	0.0625, 0.125, 0.25, 0.75
Relative potency formula	$R = 10^{\frac{\alpha_T - \alpha_R}{\beta}}$	$R = 10^{\frac{\alpha_T - \alpha_R}{\beta}}$
Reference curve suitability criteria	95% confidence interval (CI) of slope, intercept, root mean square error	95% confidence interval (CI) of slope, intercept, root mean square error
Test sample curve acceptance criteria (equivalency to reference)	95% CI difference in intercepts of test and reference curves	95% CI difference in slopes of test and reference curves
Mean % Relative Potency for each Reference	100.3%	102.4%
Precision of the assay (% Relative Potency 95% CI of the reference)	73.2-137.6%	72.9-143.7%

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In vitro / in vivo correlation

- **Goal:**

  Determine whether *in vitro* relative potency correlates to changes in the CMV pDNA vaccine-mediated immune response

  - *In vitro* % Relative Potency (RP)
    - % response relative to a reference, using RT-PCR
  - *In vivo* Immune Response in Mice
    - Anti-gB antibodies by ELISA
    - pp65 T-cell responses using IFN-γ ELISPOT

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In vitro / in vivo correlation

- **Method:**

  Evaluate hypo-potent CMV vaccine (80°C heat-degraded) versus the 100% potent CMV vaccine within the linear range of the *in vitro* and *in vivo* assays' dose-response curves

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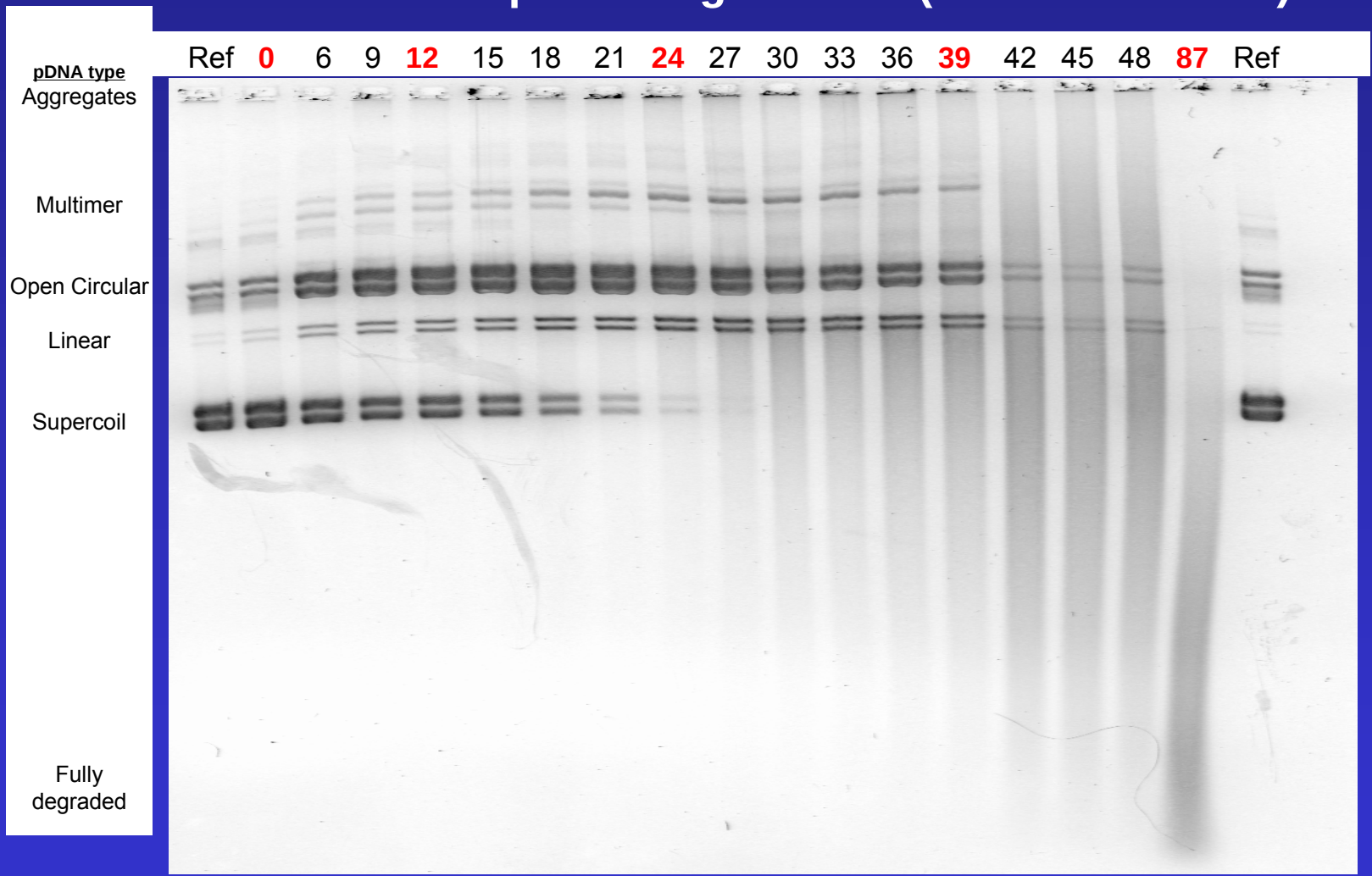
In vitro / in vivo correlation study

80°C forced pDNA degradation (hrs of treatment)



In vitro / in vivo correlation

In vivo study design: VCL-CB01

% Relative Potency (formulation treatment)	Total Dose (µg)	Total Volume (µL)	# of animals/group
~100% (0 hr at 80ºC)	10	100	11
~55% (12 hr at 80ºC)	10	100	11
~22% (24 hr at 80ºC)	10	100	11
~11% (39 hr at 80ºC)	10	100	11
~0% (87 hr at 80ºC)	10	100	5

Bilateral intramuscular injection targeted to the rectus femoris of the quadriceps were administered on Days 0 and 14 of the study

Blood was collected from each animal prior to the first injection (Baseline pre-bleed) and on Day 26 via orbital sinus puncture.

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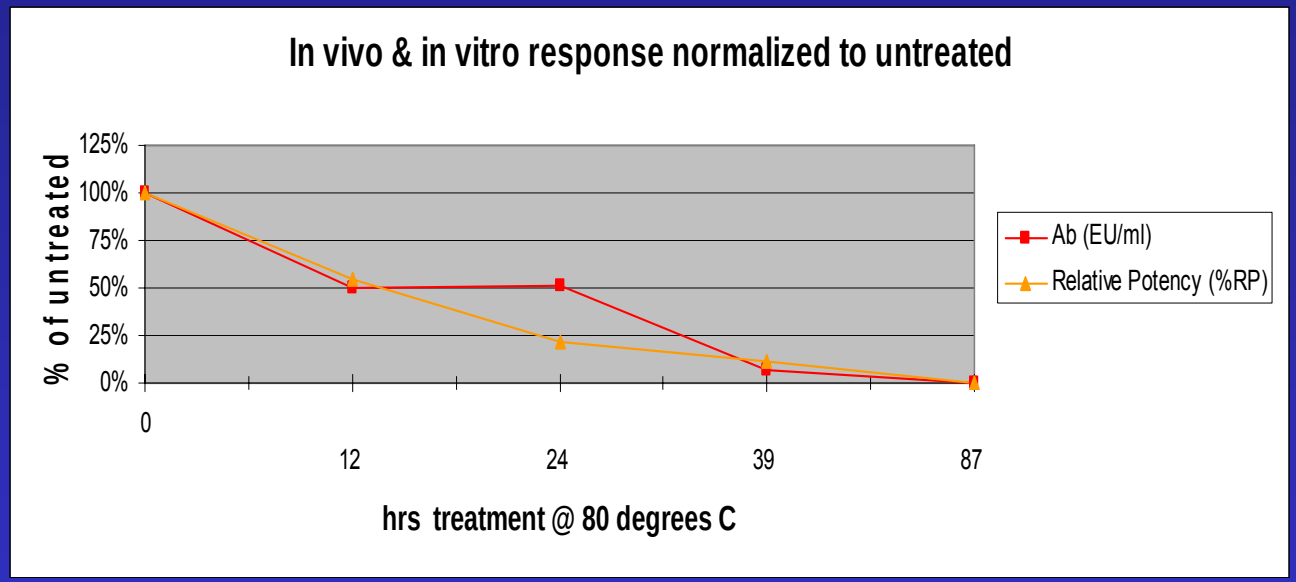
In vitro / in vivo correlation study Ab results

Normalized Results



Time @ 80°C	Relative Potency (%RP)	Anti-gB Ab (EU/ml)
0 hr	101.25	60,227 +/- 6,157
12 hr	55.1	30,402 +/- 3,504
24 hr	22.25	30,535 +/- 4,942
39 hr	11.25	4,081 +/- 2,737
87 hr	0	0

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In vitro / in vivo correlation study
T-cell results

- High variability
- Low responses
- Inconclusive results

In vitro / in vivo correlation

- Conclusions
  - Forced degradation of pDNA correlates with a drop in relative potency (RP) by RT-PCR
  - Drop in RP correlates to drop in CMV-mediated immune response
    - Antibody data appears to correlate best with RP and degradation
      - Slope Analysis of downward trend statistically significant (p=0.001)
    - ELISPOT assay - inconclusive
      - Variability too high
      - Response lower than historical data

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Summary: Characterization & Lot Release

Nucleotide structure

Characterization	Lot release
Genetic stability	Total size
	Restriction enzyme digest
	HPLC analysis (%SC, %OC, % linear)

Transcription

Characterization	Lot release
	RT-PCR
Pre-clinical immunogenicity	***% Relative potency***
In vitro expression (Immunoppt &/or Immunoblot)	

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Acknowledgements

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