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A Probabilistic Model for Risk Assessment of Residual Host Cell DNA in Biological Product

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Anxiety Attack

The Scream, by Edvard Munch, 1893

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The Problem

FDA Complete Response Letter, Question #1:

According to your DNA size distribution analysis summarized in Table 7.3.4.5.20-1, MEDI-XXX contains a significant amount of residual MDCK host cell DNA greater than 500 bp in length. This may increase the risks of oncogenicity and infectivity of MDCK DNA. As recommended in our FDA guidance, "Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases", the median size of residual DNA should be 200 bp or smaller

The Problem (Cont'd)

FDA Complete Response Letter, Question #1:

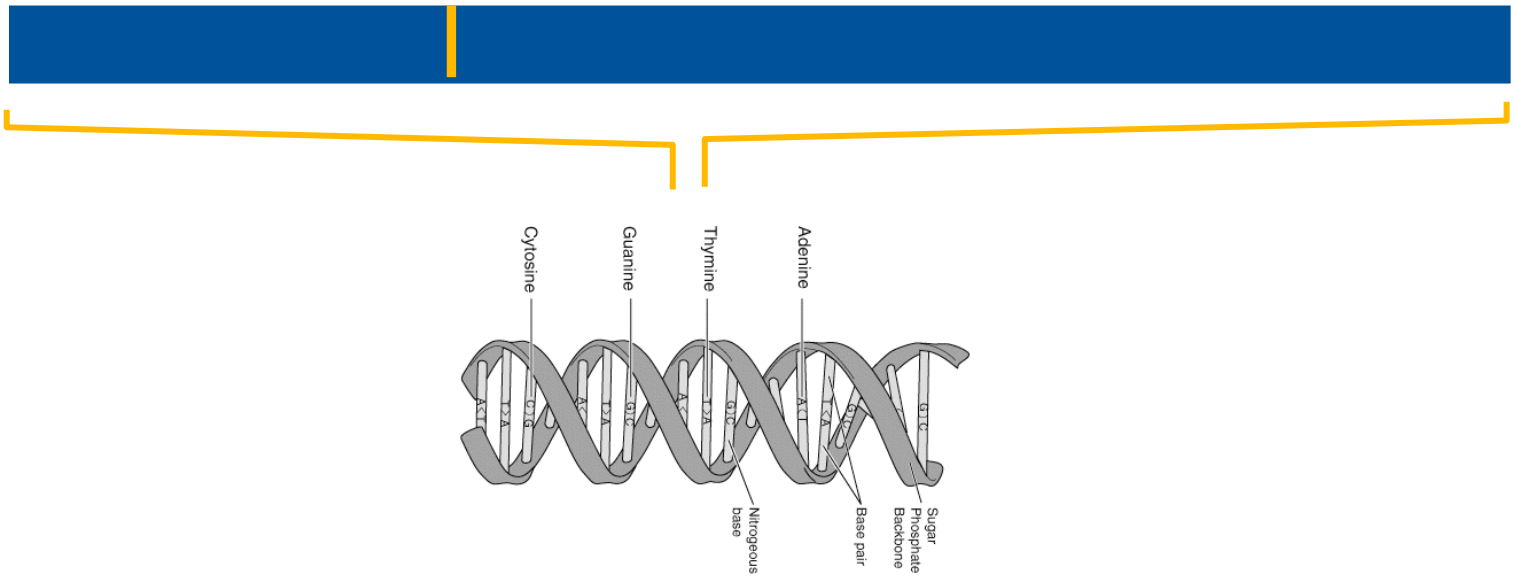
According to your DNA size distribution analysis summarized in Table 7.3.4.5.20-1, [illegible] a significant amount of residual M[illegible]reater than 500 bp in length. This may in[illegible]ncogenicity and infectivity of MDCK D[illegible]ed in our FDA guidance, "Character[illegible]tion of Cell Substrates and Othe[illegible]Materials Used in the Production of Vira[illegible]revention and Treatment of Infectio[illegible]dian size of residual DNA should be 200 bp or smaller

The Product in Question Is A Cell-based Influenza Vaccine

- It has many advantages over egg-based product
  - Unaffected by a circulating poultry pathogen
  - Easy to scale up production

But It Comes with A Price: Residual Host Cell DNA May Contain Oncogenes

Oncogene



Host cell DNA

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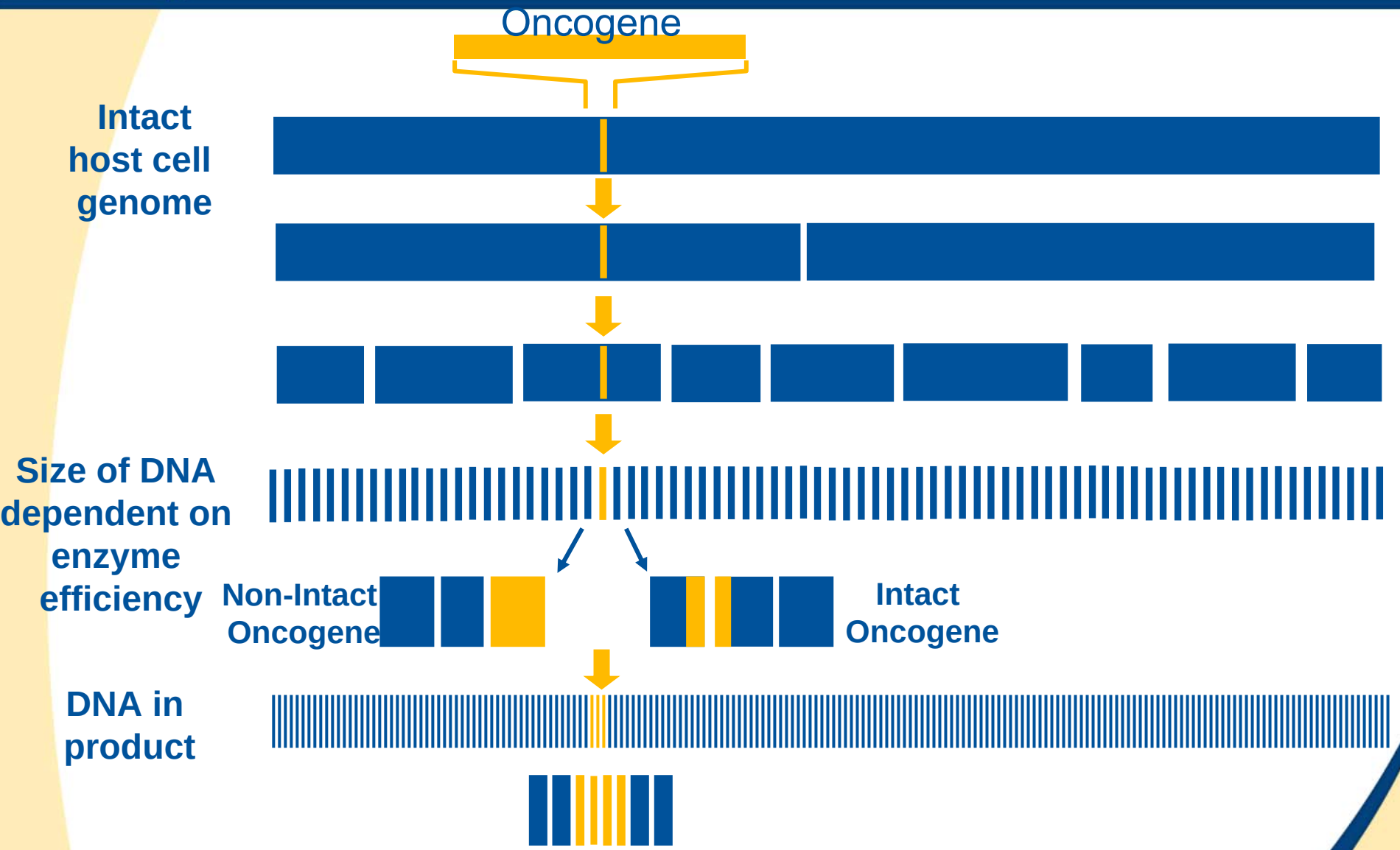
Oncogene is an enemy

- Cellular DNA can induce cancers
- Controlled by reducing quantity and size of residual MDCK DNA in final product

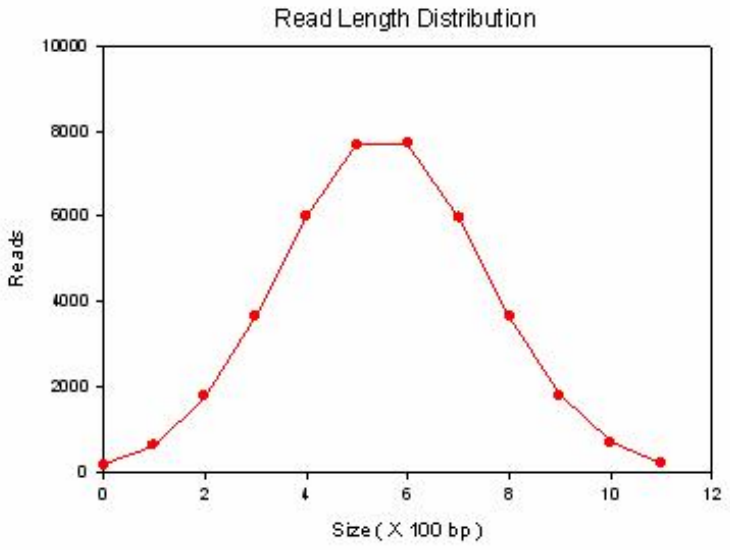
Enzymatic Degradation Inactivates DNA

Benzonase and other ingredients

Enzyme digests genomic DNA, rendering it inactive



Efficiency of Current Process



Oncogene Average Coding Region (1925 bp)

Median Size in Vaccine (450 bp)

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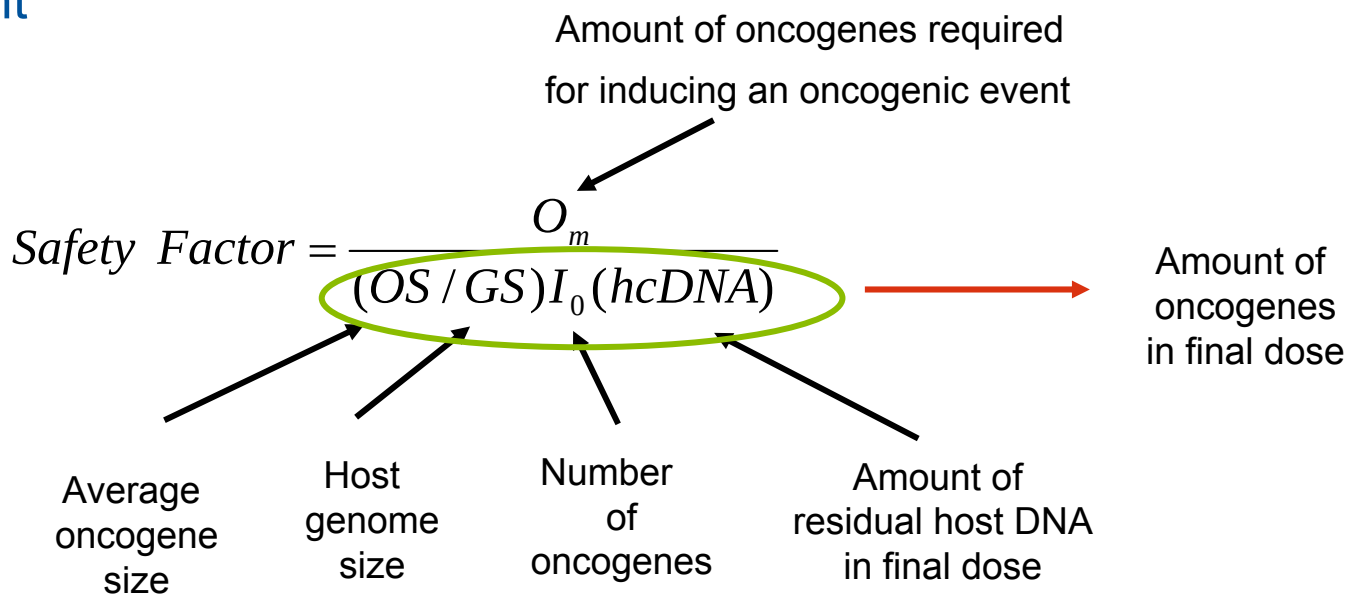
FDA Concerns

- FDA CRL question
  - FDA regulation requires mitigating risks by reducing residual DNA to a median size ≤**200** bp
  - Our process can only achieve a median size of **450** bp
- Risks of oncogenicity and infectivity may be increased

Risk Assessment

- Safety factor
  - Number of doses taken to induce an oncogenic or infective event



K. Peden et al. (2004), Vaccine Cell Substrate. and L. Sheng et al. (2008), Biologicals.

Safety Factor per FDA-recommended Method

- If cellular DNA contained an active oncogene it would take over 11.6 billion doses to cause an oncogenic event
  - If 250 million doses of vaccines are used annually, in less than 46.4 years one oncogenic event may be observed

O_m (ng)	9400*
OS	1950
GS	2.41E+09
I_0	1
hcDNA (ng)	1
Safety Factor	1.16E+10

* Oncogenic dose derived from mouse

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What Should We Do?

Oncogenic risk is overstated

- The denominator includes amount of fragmented oncogenes

Amount of oncogenes required for inducing an oncogenic event

$$Safety\ \ Factor = \frac{O_m}{(OS/GS)I_0(hcDNA)}$$

Average oncogene size

Host genome size

Number of oncogenes

Amount of residual host DNA in final dose

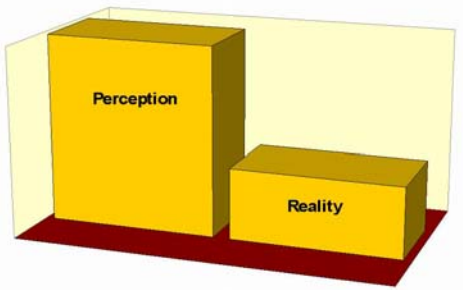
Amount of oncogenes in final dose

=

Amount of **unfragmented** oncogenes in final dose

+

Amount of **fragmented** oncogenes in final dose

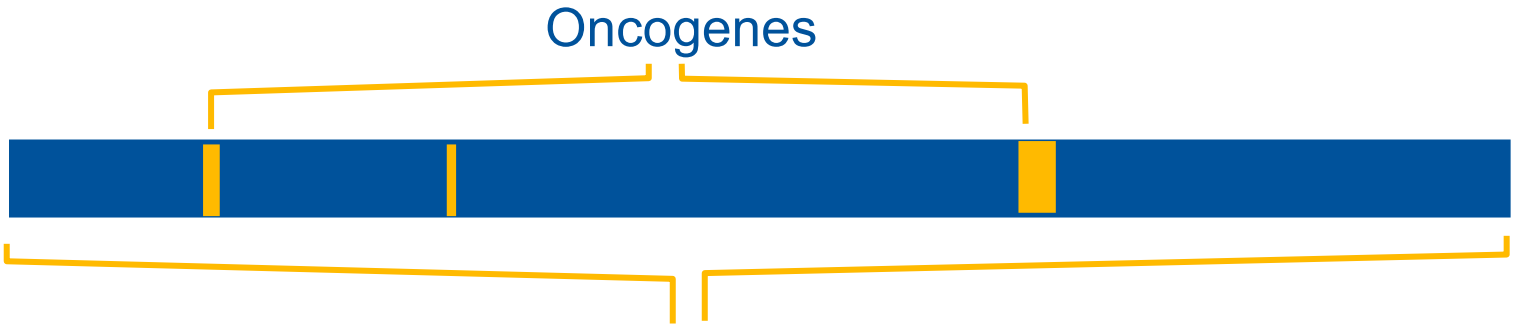


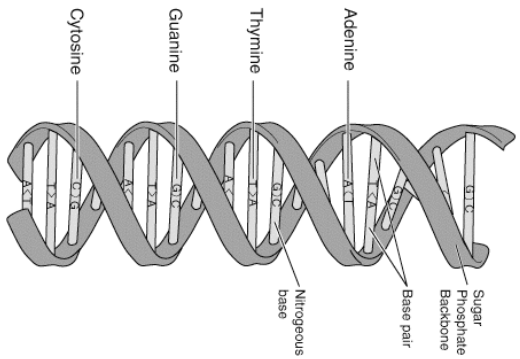
Issues with FDA-recommended Method

- It does not take into account DNA inactivation

Issues with FDA-recommended Method (cont'd)

- It does not account for sizes of oncogenes





Host cell DNA

$$Safety\ Factor = \frac{O_m}{(OS/GS)I_0(hcDNA)}$$

Average oncogene size

Host genome size

Number of oncogenes

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Hope

- This finding gives us hope that with median residual DNA size of 450 bp (albeit not quite up to the regulatory bar of 200 bp) perhaps the oncogenicity and infectivity risks are already reduced to an acceptable level.

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Negotiation with FDA

- Standard method overestimates risk
- If DNA inactivation step is incorporated in the calculation, the risk might be tolerable

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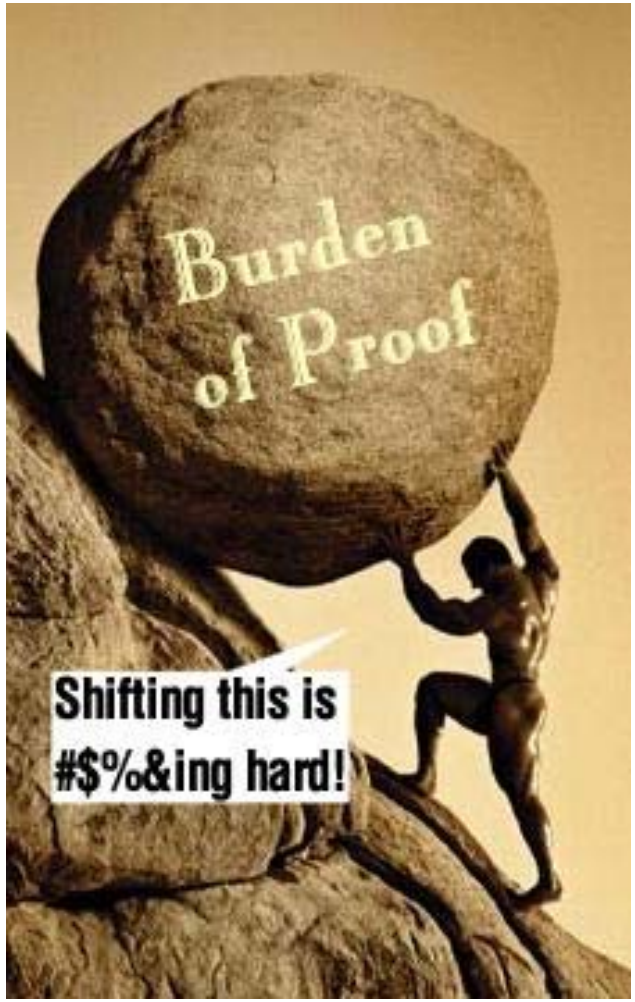
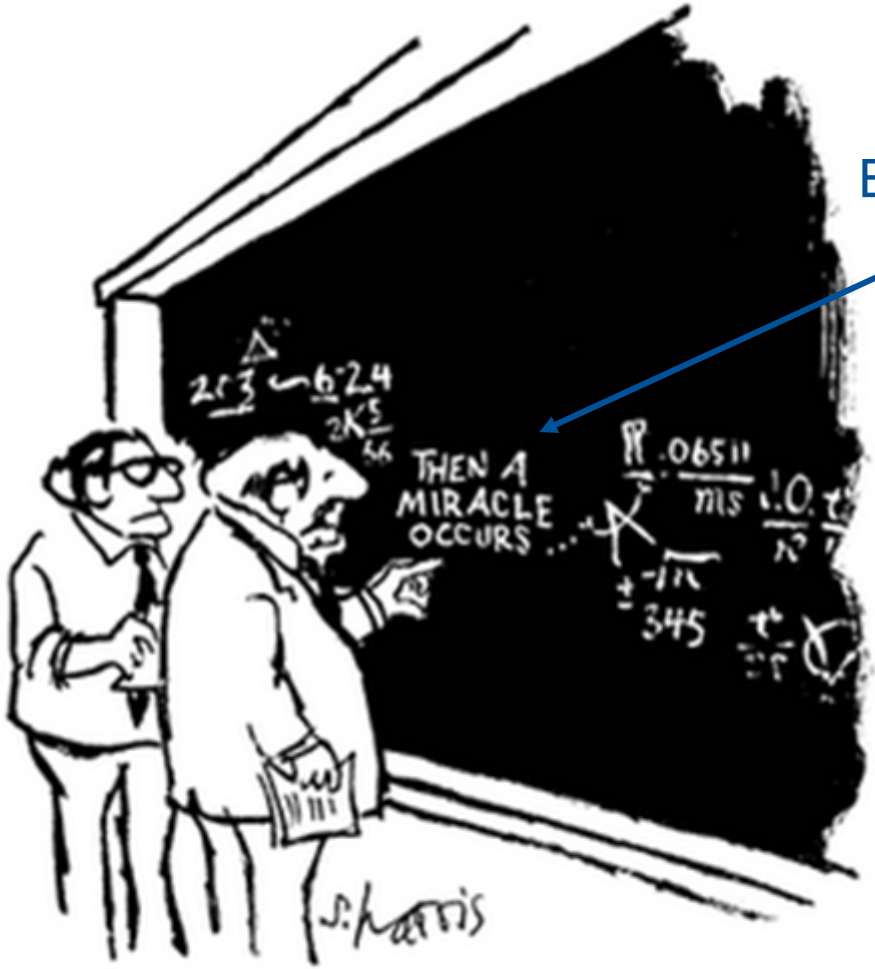
Burden of Proof



How to Incorporate DNA Inactivation in the Risk Assessment?



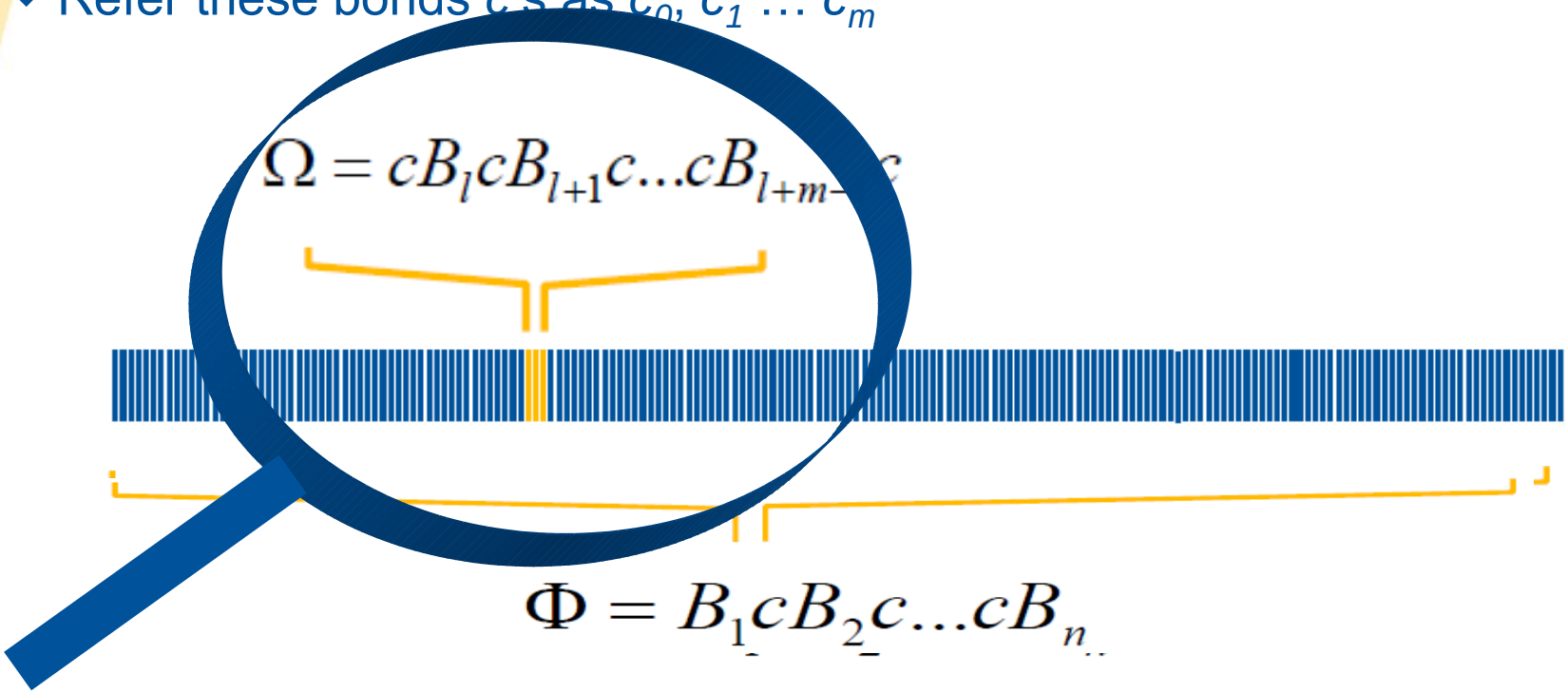
"I think you should be more explicit here in step two."

Enzymatic degradation of DNA

Source: http://1.bp.blogspot.com/_vgEA7CHGLe8/SzIAZHWs-vI/AAAAAAAAAVc/vZcmDlRlxSY/s320/miracle.gif

Model of DNA Inactivation Process

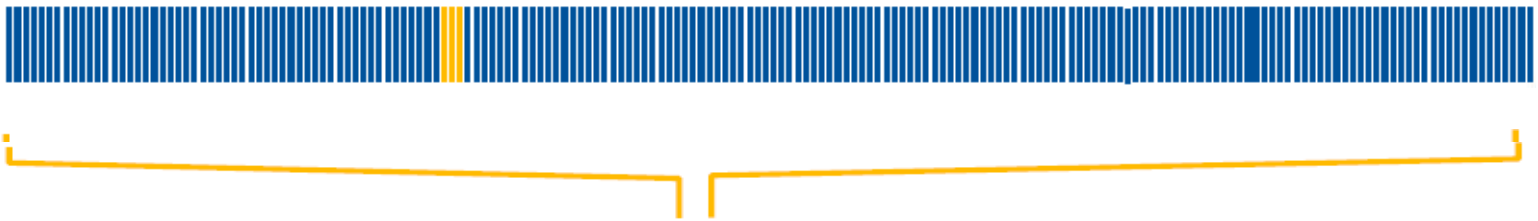
- Notation
  - Φ, Ω, c denote the entire genomic DNA, oncogene and bond
  - Refer these bonds c's as c_0, c_1 … c_m



Model of DNA Inactivation Process (Cont'd)

- Model
  - Define x_i as random variables
    - > $P[x_i = 1] = P[c_i \text{ is cut by enzyme}] = 1 - P[x_i = 0] = p$
    - > x_i are *i.i.d.* according to a Bernoulli distribution.

$$\Omega = cB_l cB_{l+1} c \ldots cB_{l+m-1} c$$

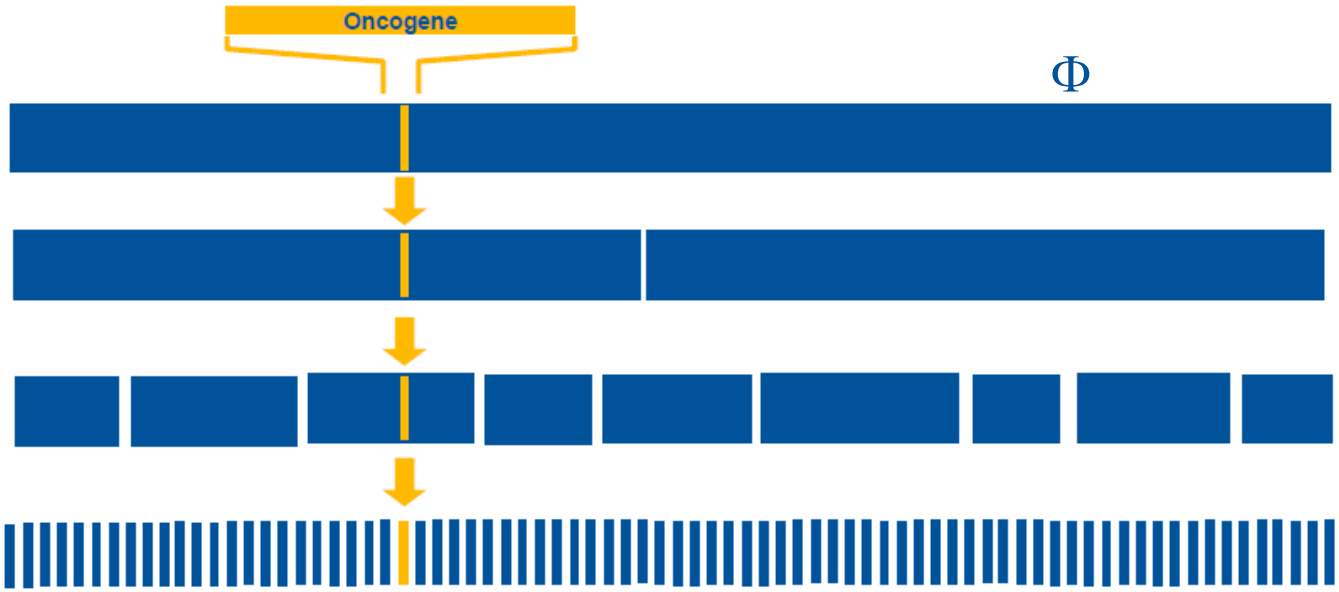


$$\Phi = B_1 cB_2 c \ldots cB_n$$

Model of DNA Inactivation Process (Cont'd)

- Probability for a partial digestion of Φ to contain oncogene Ω
  - $P[x_1 = x_2 \ldots = x_{m-1} = 0] = (1 - p)^{m-1}$.

$$\Omega = B_l c_1 B_{l+1} c_2 \ldots c_{m-1} B_{l+m-1}$$



Partial digestion of Φ

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Estimation of Safety Factor

- Safety factor (SF)
  - Number of doses taken to induce an oncogenic or infective event

$$\sum_{j=1}^{SF} Y_j = O_{m.}$$

Y_j - No. of unfragmented oncogenes in dose j, $j = 1,\ldots, SF$.

  - By The Large Number Theory

$$\frac{\sum_{j=1}^{SF} Y_j}{SF} \approx E[Y]. \quad \text{or} \quad SF \approx \frac{\sum_{j=1}^{SF} Y_j}{E[Y]}$$

  - E[Y] can be derived using conditional probability calculations

Expected Value of No. of Unfragmented Oncogenes in A Dose

$$E[Y] = \sum_{i=1}^{I_0} (1-p)^{m_i - 1} \frac{m_i}{M} E[U]$$

I_0: Number of oncogenes in host genome
p: Enzyme cutting efficiency
m_i: Oncogene sizes
M: Hose genome size
E[U]: Expected amount of residual hose DNA/dose

Proof of the Theoretical Result

- Trust me!

Estimation of Safety Factor (Cont'd)

- Safety factor

Amount of oncogenes required for inducing an oncogenic event

$$SF = \frac{O_m}{\sum_{i=1}^{I_0} (1-p)^{m_i - 1} \frac{m_i}{M} E[U]}.$$

Expected amount of unfragmented oncogenes in a dose

Standard method is a special case: p = 0

- Safety factor

Amount of oncogenes required for inducing an oncogenic event

$$SF = \frac{O_m}{\sum_{i=1}^{I_0} (1-p)^{m_i - 1} \frac{m_i}{M} E[U]} = \frac{O_m}{\sum_{i=1}^{I_0} \frac{m_i}{M} E[U]} = \frac{O_m}{(OS/GS) I_0 E[U]}$$

Average oncogene size

Host genome size

How to estimate enzyme cutting efficiency p?

- Impacted by many factors
- No direct method to measure it

Modeling Length of DNA Segment

- After enzyme digestion, any DNA segment takes the form

$$B_1 c B_2 c \ldots c B_X$$

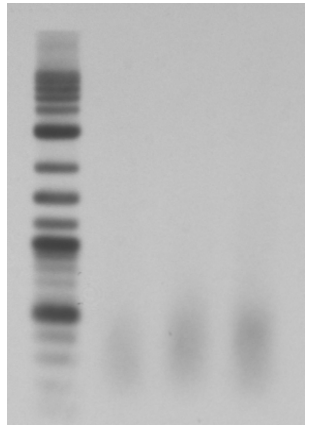
Length X, random variable

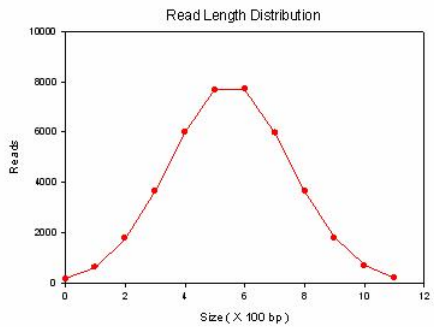
- Let p denote the probability for enzyme to cleave bond c. Thus X has properties
  - Represents number of trials until the first cut
  - Follows a geometric distribution with parameter p,
    - > $Prob[X=k]=(1-p)^{k-1}p$
    - > $Median = -\dfrac{\log 2}{\log(1-p)}$

Experimental Determination of Residual DNA Distribution

- DNA size quantified thru electrophoresis
- Distribution of DNA length estimated, median size determined

$$Median(DNA) = Med\ (= 450\ bp)$$





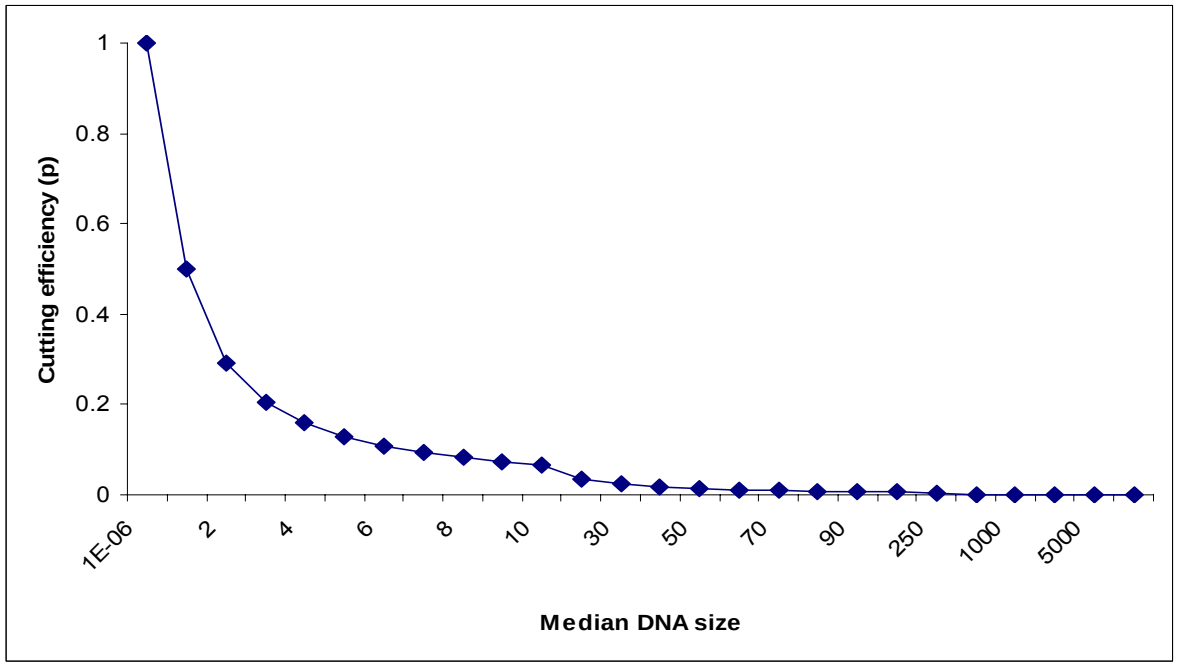
Connecting Dots

"Please join us."

Estimation of Enzyme Efficiency

- Equate theoretical median to its estimate to get an estimate of enzyme efficiency *p*

$$-\frac{\log 2}{\log(1-p)} = Med \quad \Rightarrow \quad \hat{p} = 1 - 2^{-\frac{1}{Med}}$$



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Safety Factor

- If cellular DNA contained an active oncogene it would take over 234 billion doses to deliver the oncogenic dose used in the mouse studies

O_m (ng)	9400
Oncogene size	1950
MDCK genome size	2.41E+09
Median	450
hcDNA (ng)	1
Safety Factor	2.34E+11

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Oncogenic Risk Comparison

- FDA method overestimates oncogenic risk by 19-fold
- Reducing residual DNA with median size of 450 bp is adequate to mitigate oncogenic risk

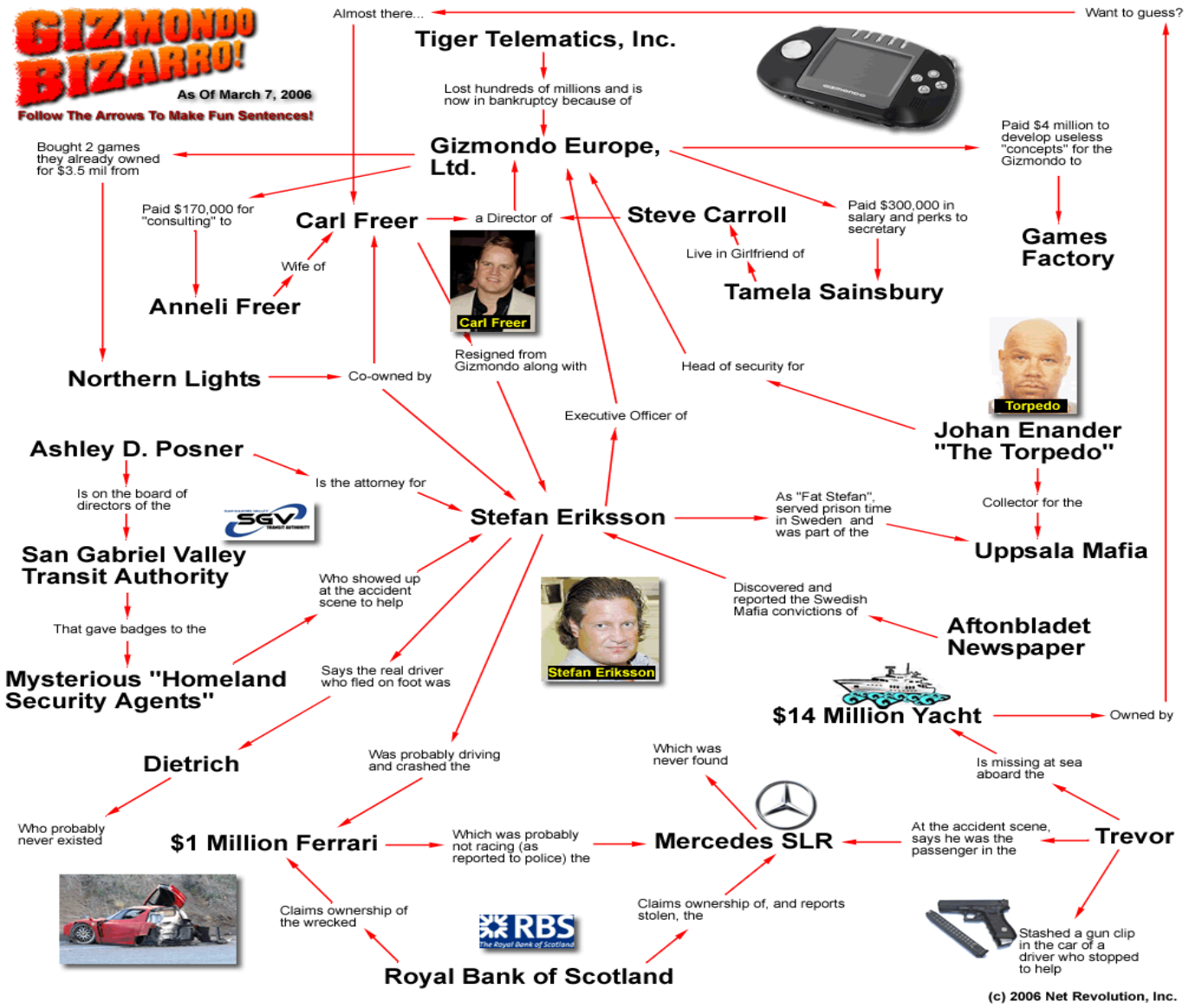
Our Method

O_m (ng)	9400
Oncogene size	1950
MDCK genome size	2.41E+09
Median	450
hcDNA (ng)	1
Safety Factor	2.34E+11

FDA Method

O_m (ng)	9400*
Oncogene size	1950
MDCK genome size	2.41E+09
I_0	1
hcDNA (ng)	1
Safety Factor	1.16E+10

Pre-VRBPAC Meeting – Too Complicated To Explain



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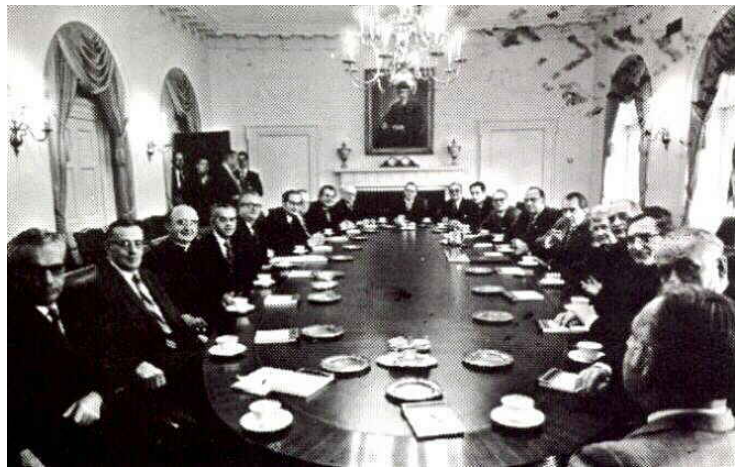
VRBPAC Advisory Committee Meeting

- VRBPAC meeting took place on September 25, 2008, Silver Spring, Maryland
- A slim-down version of the method used
- “Great job!” commented Dr. Peden, Director of Vaccine Evaluation, from the FDA



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Conclusions

- A probabilistic model proposed to allow for quantification of risks of oncogenicity and infectivity due to residual DNA
- The method takes into account DNA inactivation steps in manufacturing process and biological nature of host cell genome and potential oncogenes and infective agents
- It renders more precise and accurate assessment

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Conclusions (Cont'd)

- It's a great example of collaboration and teamwork!

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Acknowledgement

- Lanju Zhang
- Mark Galinski
- Ryan Yamagata

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