FDA U.S. FOOD & DRUG ADMINISTRATION
NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Division of Genetic and Molecular Toxicology

Robert H. Heflich, Director

Mugimane G. Manjanatha, Deputy Director

Current DGMT Staff

- Government Positions — 27 full time employees
  - Research Scientists, Staff Fellows & Visiting Scientists : 14
  - Support Scientists : 10
  - Administrative : 2
  - FDA Commissioner Fellows: 1

- ORISE Post Docs, Graduate Students, etc.:
  - 4 ORISE Postdocs (one externally supported)

- Total = 31

DGMT Principal Investigators

Investigator	Standard assay/research projects/support
Xuefei Cao	In vitro human tissue models/CTP, NTP
Tao Chen	Ames test/genomics, nano, NGS/CTP
Vasily Dobrovolsky	*Pig-a*, in vivo and in vitro models/CTP
Wei Ding	Comet/immunotox/OWH
Xiaoqing Guo	MLA/quantitative methods, in vitro human/CTP
Bob Heflich	in vivo-in vitro MN/in vivo models, in vitro human models/CTP
Manju Manjanatha	Comet, in vivo-in vitro TGR/ assay development/CRADA
Page McKinzie	Cancer driver mutation, NGS
Nan Mei	MLA/quantitative methods, nano, assay development/CTP
Meagan Myers	Cancer driver mutations/OWH
Barbara Parsons	Cancer driver mutations/CRADA
Javier Revollo	NGS, *Pig-a*, gene editing/CTP
Patrice McDaniel	*Pig-a* database

Outreach

- Collaborations
  - NCTR Divisions: DBT, DSB, DBT, OSC
  - FDA regulatory Centers and Offices: CTP, CDER, CDRH, CBER, CFSAN, OWH
  - Government agencies: NTP, NIEHS
  - Others: UMD, UAMS, Harvard, UALR, TERA, Litron, Covance, BioReliance, Charles River , IIVS

- Global leadership outreach
  - Organizations: OECD, International Workshop on Genotoxicity Testing (IWGT), ILSI/HESI
  - Others: St. George's College (London), NIHS (Japan), Swansea Univ (UK), Teijin Pharma (Tokyo), Osaka Medical Center (Japan)

DGMT Mission (Vision)

Improve public health by 1) providing the Agency with the expertise and tools necessary for comprehensive assessment of genetic risk and by 2) strengthening approaches to integrate knowledge of genetic risk into regulatory decision making.

DGMT Goals

- Respond to Agency needs for chemical-specific data (e.g., nanomaterials, drug impurities, tobacco products) and assay expertise (e.g., CDER PTCC)
- Maintain DGMT's tradition of leadership in regulatory assay development and validation (Historical: MLA, *Hprt*, TGR; New: *Pig-a,* EpiComet Chip, Hairless Albino TGR): active in OECD, IWGT, ILSI/HESI and NTP assay development projects
- **Establish new paradigms for regulatory decision making that integrate measures of genetic risk with biomarkers of toxicity**

Strategies for Establishing New Paradigms for Regulatory Decision-Making

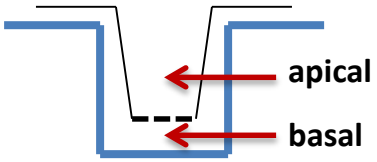
- Develop better **biological models** for assessing human risk that integrate genotoxicity with other measures of toxicity
- Develop more comprehensive approaches for monitoring **genetic variation**
- Develop better ways of **evaluating data** to determine human risk

Strategy: Develop Better Biological Models

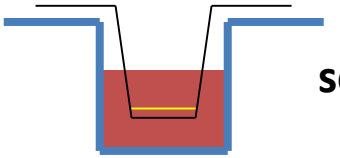
- Historically, genetox assessments have been conducted in bacteria, mammalian cell lines, and inbred (transgenic) rodents. Models that better represent human functions would increase value of data for evaluating risk.
- Current projects:
  - **Human (and rodent) in vitro organotypic models (E7549, E2200, E7623)**
  - High-content/medium-throughput screening approaches using metabolically competent human cell lines and primary cells (E7608, E7609, C18001)
  - 3D tumor models (7551)
- Proposed/in-development projects:
  - *In vitro* human, rodent, and alternative (*C. elegans*) germ cell models (C16037)
  - Adapting the *Pig-a* assay to measure mutation in male germ cells

Establishing Human ALI Airway Models

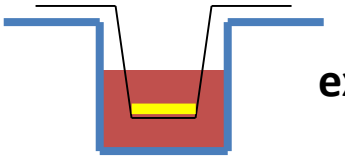
Side view of the insert



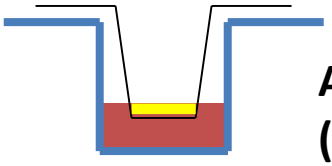
Costar inserts



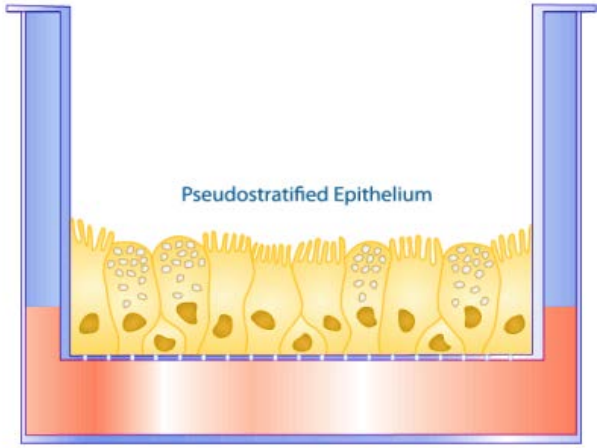
seeding



expanding



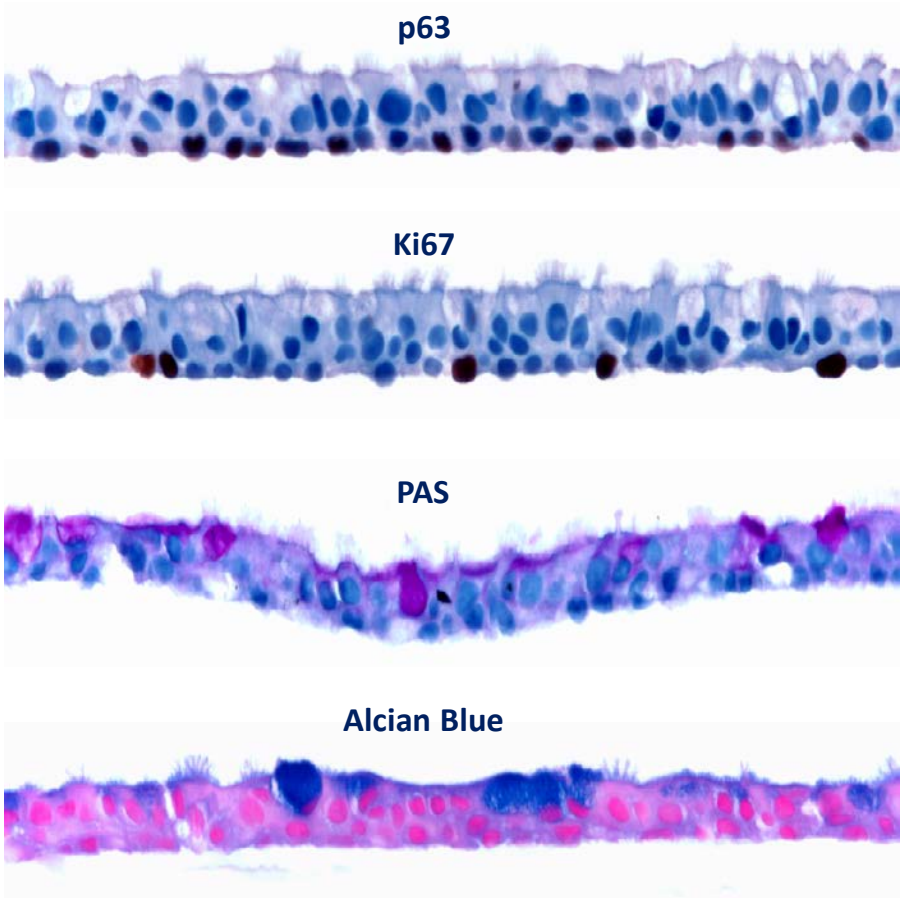
Air-lifting (air-liquid-interface)



Air-Liquid Interface Culture

- **Pseudo-stratified structure**
- **Ciliated epithelial cells**
- **Goblet cells**
- **Basal cells**
- **Tight junctions on the apical side**

Histological Characterization of the ALI Airway Models



--- Mucus secretion

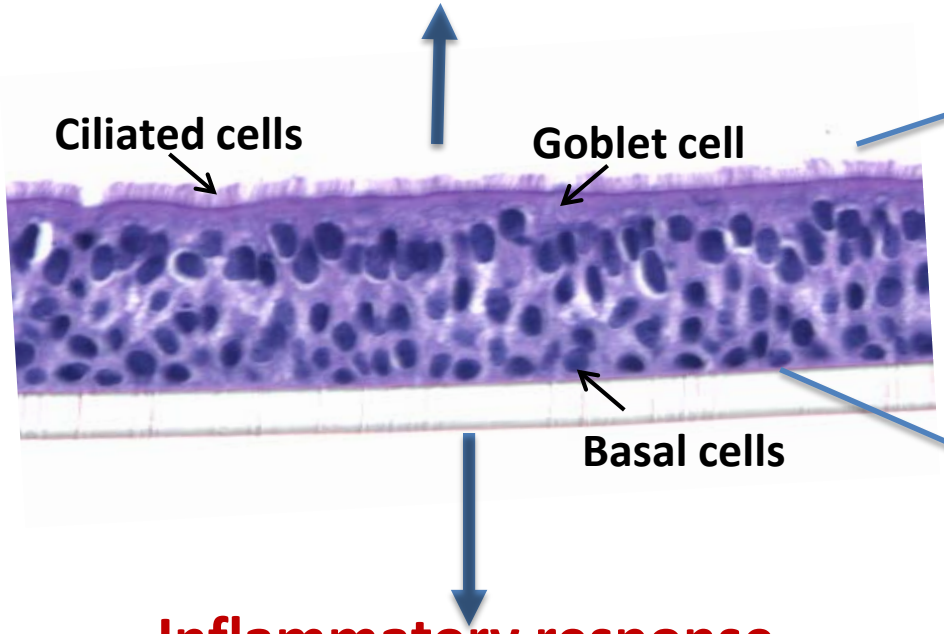
MUC5AC/5B quantification; hyperplasia of goblet cells

--- Mucociliary clearance

Cilia beating rate

--- Tissue permeability (tight junction integrity)

TEER measurement; immunofluorescence staining of tight junction markers



Metabolism

Phase I & II metabolism

Tissue structure changes

Squamous metaplasia, epithelial hyperplasia

DNA Damage

Comet

--- Inflammatory response

Cytokine release; NF-kB activity

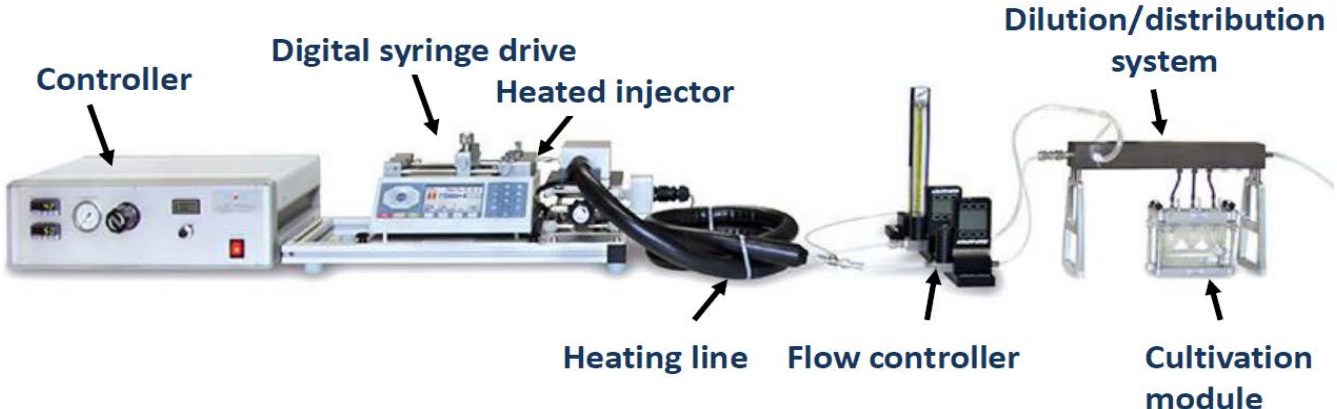
--- Modification of extracellular matrix

MMP activity; biomarkers of extracellular matrix regeneration

Tissue Exposure Systems

- Whole cigarette smoke system (CTP)
- Aerosol exposure

- Vapor/gas exposure



Projects

- Ongoing
  - E7549: Evaluating the toxicity and inflammation produced by cigarette smoke using human *in vitro* airway models (CTP)
  - E2200: Developing an *in vitro* system to evaluate the disease-related toxic effects of inhaled test agents in human airway tissue models (NTP)

- Proposals/preliminary studies/ideas
  - Apply **computational fluid dynamic modeling** to scale *in vivo* to *in vitro* exposures (E7603)
  - Develop **rat and transgenic rat versions** of tissue models: apply parallelogram approach to *in vivo* and *in vitro* data in rats and humans (E7603)
  - Develop **NGS sequencing** approaches for measuring mutation in tissue models—develop **TGR** models? ***Pig-a*** gene mutation?
  - **More complex** models: adding cell types, combining tissues, MPS

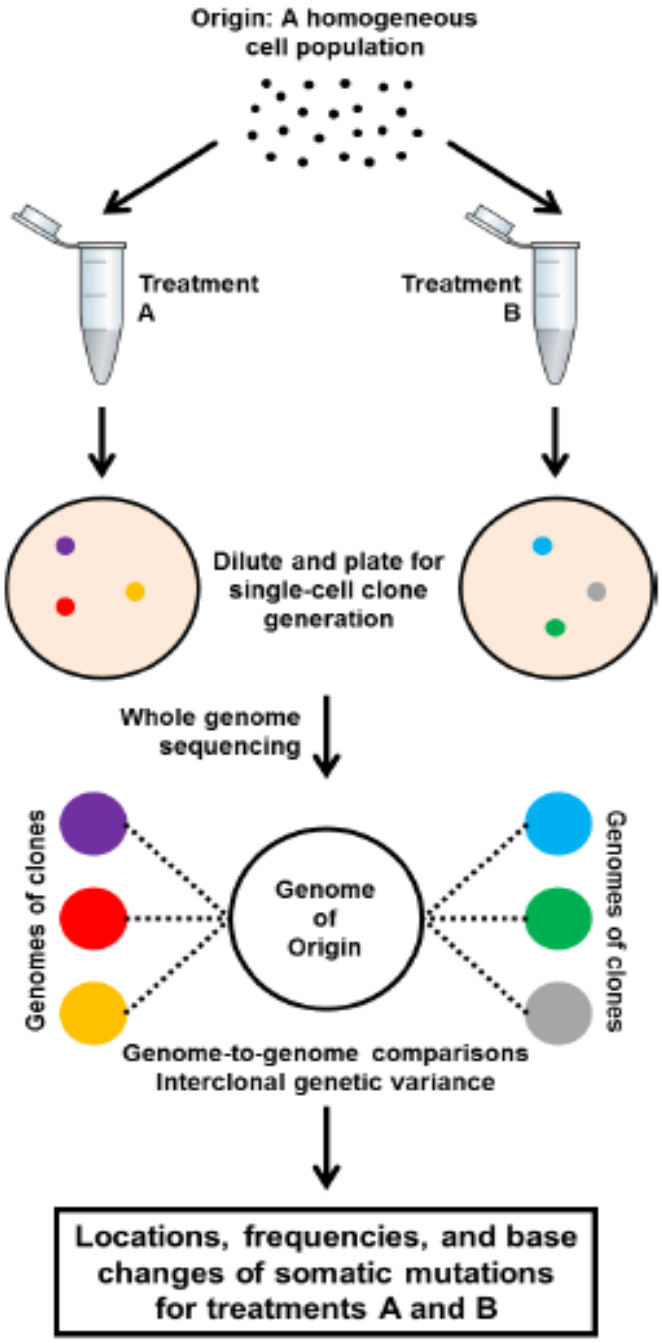
Strategy: More Comprehensive Approaches for Monitoring Genetic Variation

- **Regulatory genetox assessments rely on DNA damage (Comet), cytogenetic (chromosome breakage), and reporter gene mutation data from cell lines and inbred (transgenic) rodents—i.e., using surrogate endpoints in surrogate systems for evaluating effects in humans — and are generally limited to performing hazard ID**

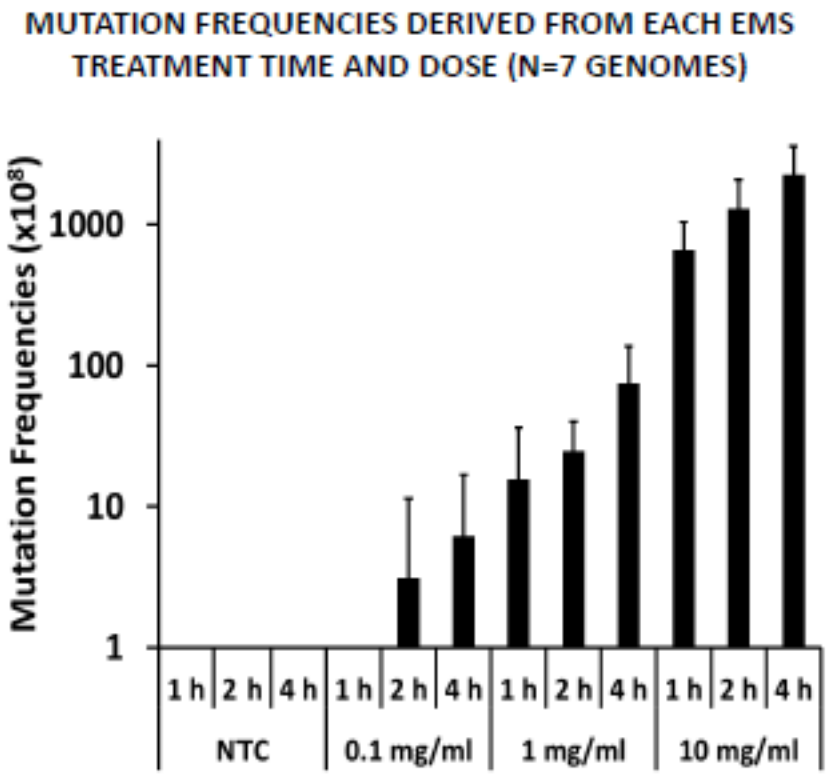
- Ongoing projects:
  - Using ACB-PCR to evaluate cancer driver mutations (E7229, E7336, E7438, E7551, E7619)
  - Using ddPCR and error-corrected NGS for evaluating cancer driver mutations (E7630)
  - Using error-corrected NGS to evaluate *Pig-a* mutation in bone marrow erythroid and granulocyte precursors (E7587)

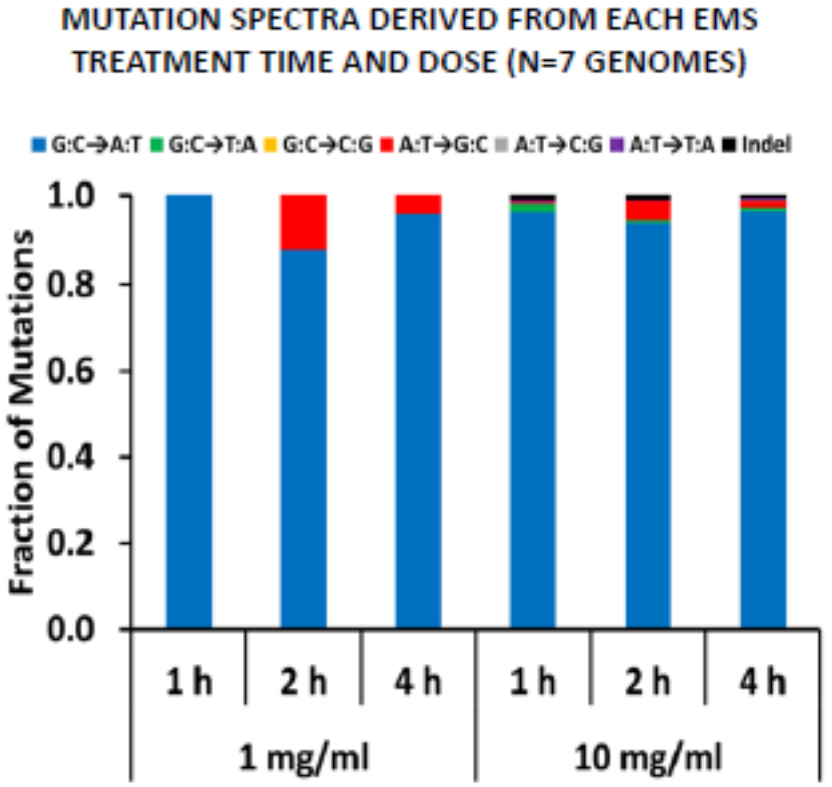
- Proposed/beginning projects:
  - Using NGS for evaluating mutation transmission through germ cells (C16037)
  - Duplex NGS for rare mutation quantification (E7629)
  - **High fidelity NGS and whole genome clone analysis (C15081)**
  - **Off-target effects of CRISPR-mediated genetic engineering in mammals (E7646)**

High Fidelity NGS and Whole Genome Clone Analysis



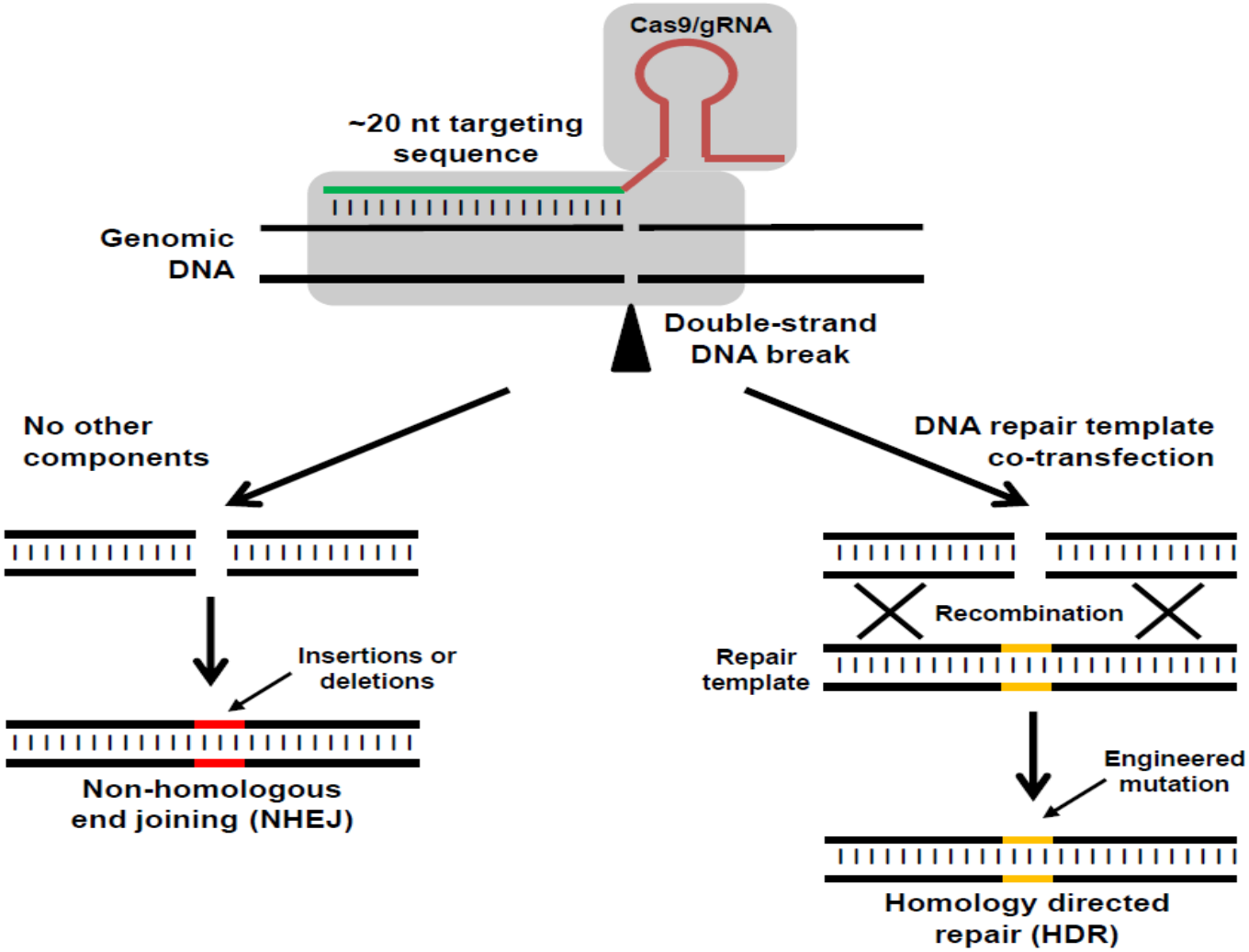
High Fidelity NGS and Whole Genome Clone Analysis



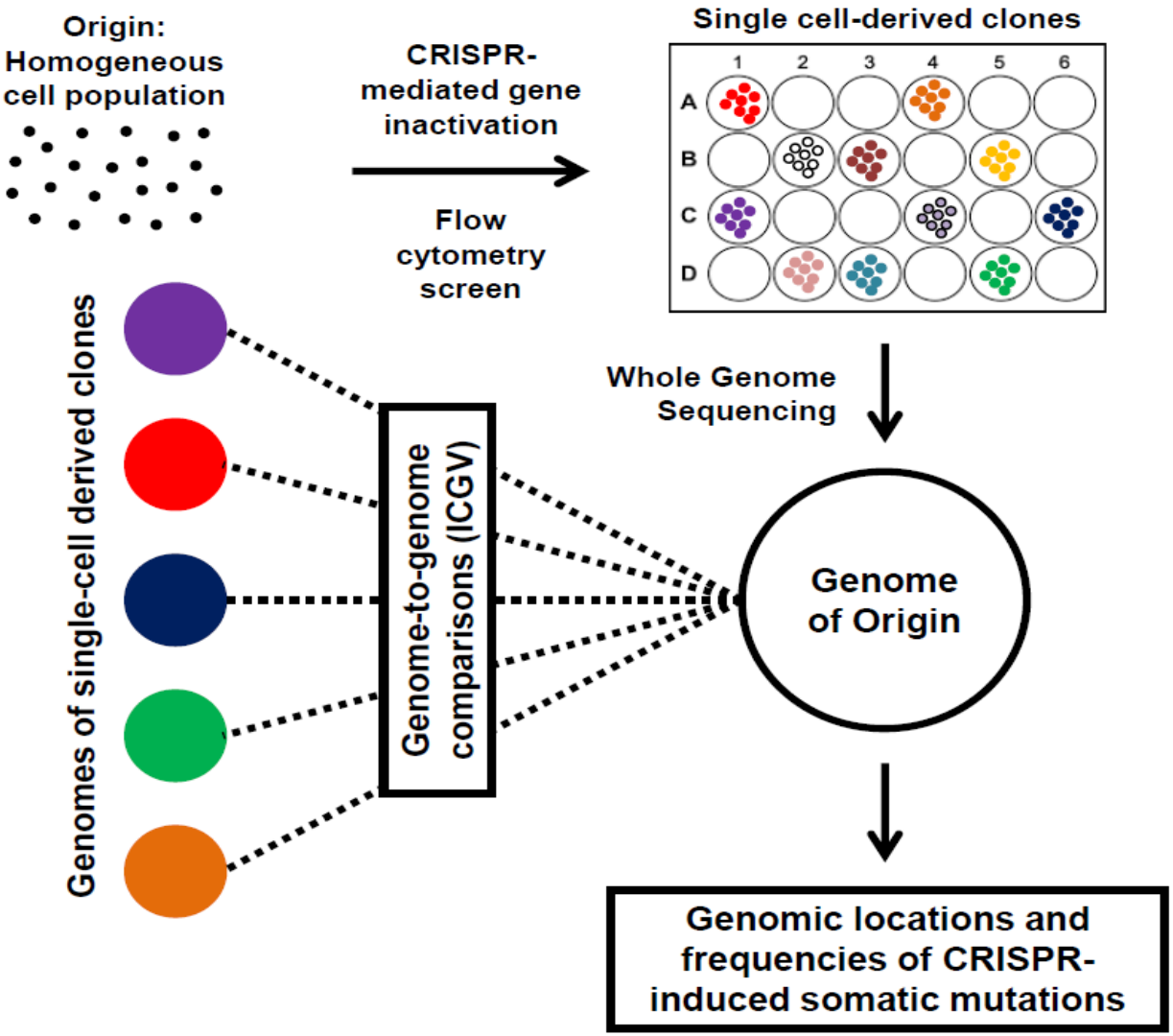


Preliminary data with *in vitro* mammalian cells (L5178Y, a cell derived from rat T lymphocytes)

Off-Target Effects of CRISPR-Mediated Genetic Engineering in Mammals



Off-Target Effects of CRISPR-Mediated Genetic Engineering in Mammals



Feedback Requested

Questions, comments?

Are we emphasizing the most productive areas for research?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