

# Regulatory divergence across mammalian species and its impact on species-specific cancer susceptibility

Health and Safety implementation for sample collection



**ARTICLE**

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## A high-resolution map of human evolutionary constraint using 29 mammals

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## Genome 10K: A Proposal to Obtain Whole-Genome Sequence for 10 000 Vertebrate Species

GENOME 10K COMMUNITY OF SCIENTISTS\*

\*GI0KOCOS authors are listed in the Appendix.

Diego Villar  
Odom laboratory  
18<sup>th</sup> July 2012

# Evolutionary comparisons as a tool to study phenotypic diversity

Large regulatory component

a) Rapid divergence of transcription factor binding

REPORTS

## Five-Vertebrate ChIP-seq Reveals the Evolutionary Dynamics of Transcription Factor Binding

Dominic Schmidt,<sup>1,2\*</sup> Michael D. Wilson,<sup>1,2\*</sup> Benoit Ballester,<sup>3\*</sup> Petra C. Schwalie,<sup>3</sup> Gordon D. Brown,<sup>1</sup> Aileen Marshall,<sup>1,4</sup> Claudia Kutter,<sup>3</sup> Stephen Watt,<sup>3</sup> Celia P. Martinez-Jimenez,<sup>5</sup> Sarah Mackay,<sup>6</sup> Iannis Talianidis,<sup>3</sup> Paul Flícek,<sup>1,7†</sup> Duncan T. Odom<sup>1,2†</sup>

b) Divergence of gene expression levels

## The evolution of gene expression levels in mammalian organs

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c) Divergence of epigenome

## Comparative Epigenomic Annotation of Regulatory DNA

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# Understanding mammalian regulatory codes will inform species-specific cancer susceptibility

- Cancer susceptibility varies widely in mammalian species
- Size and lifespan in mammals is not correlated with cancer incidence (Peto's paradox)
  - Whales or elephants have hugely decreased cancer risk *versus* mice
  - Cancer incidence is low in non-human primates
  - Species specific adaptations make some rodent species resistant to cancer
- Tissue-specific regulatory codes likely contribute to these differences

Research article

Open Access

## Comparative analysis of cancer genes in the human and chimpanzee genomes

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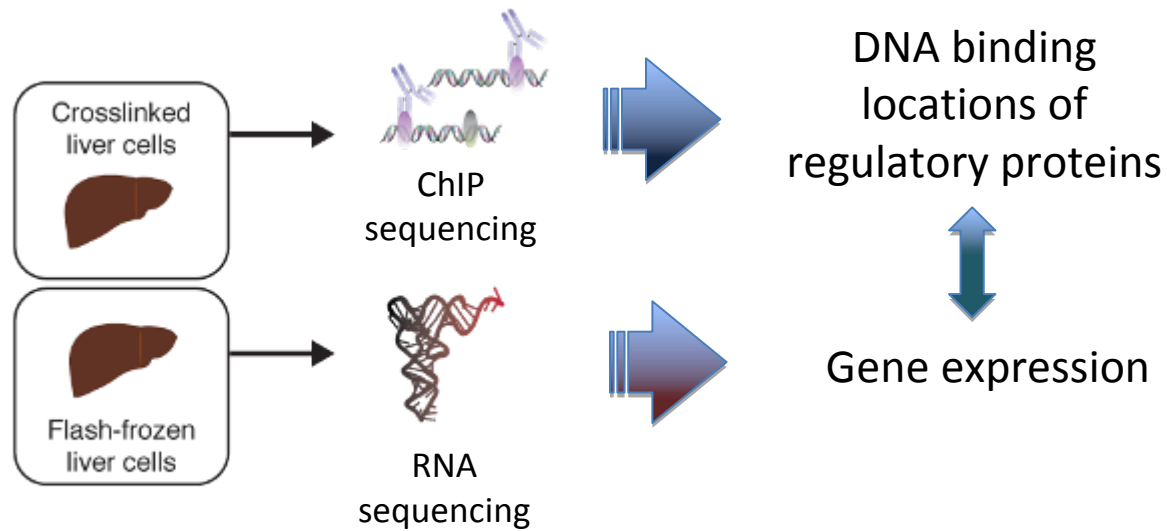
\* Corresponding author

## Resistance to experimental tumorigenesis in cells of a long-lived mammal, the naked mole-rat (*Heterocephalus glaber*)

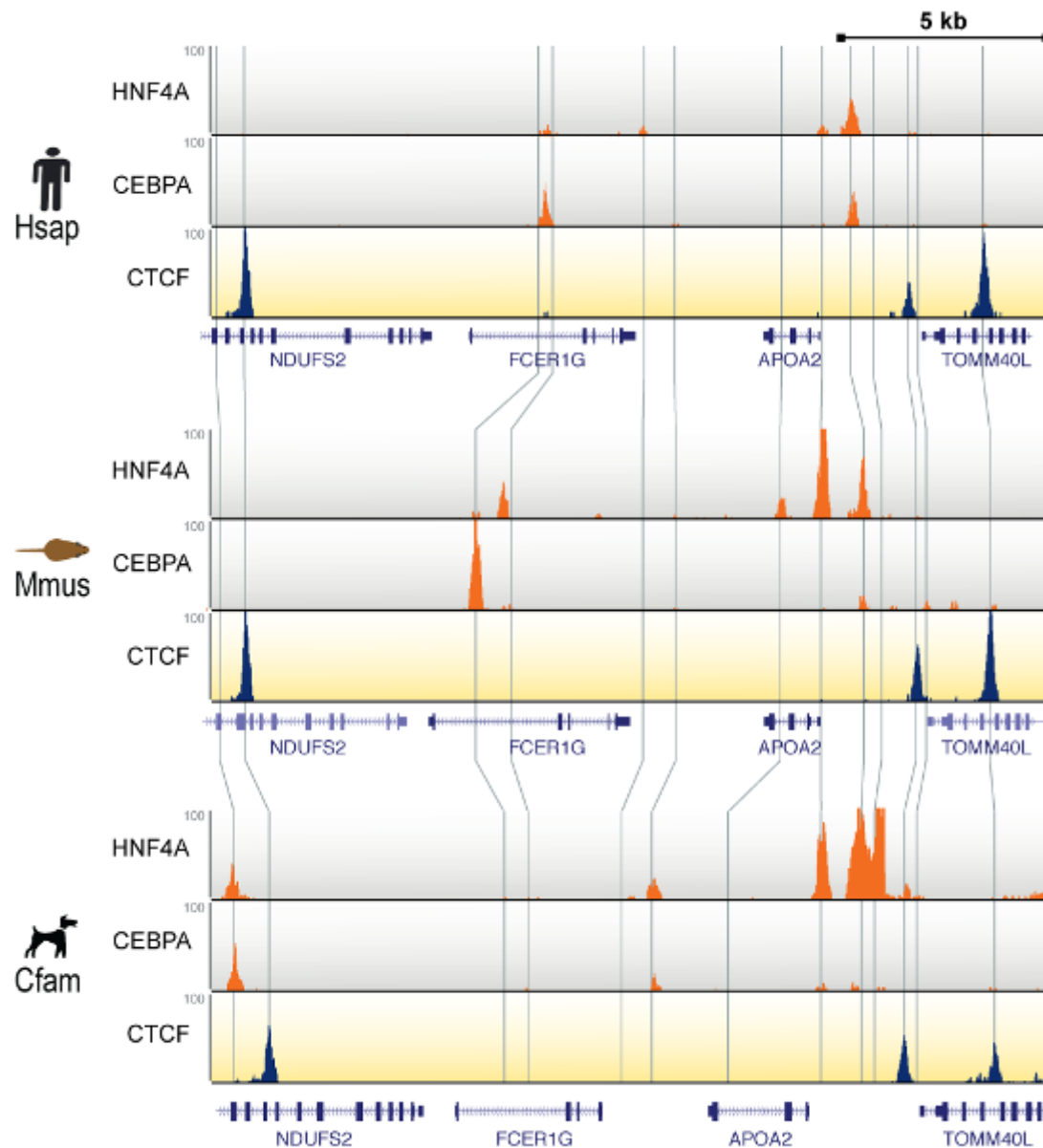
Sitai Liang, James Mele, Yuehong Wu, Rochelle Buffenstein and Peter J. Hornsby

Key words: naked mole-rat; longevity; cancer; oncogenes; crisis; DNA damage.

# High-throughput sequencing and comparative genomics in mammalian liver tissue



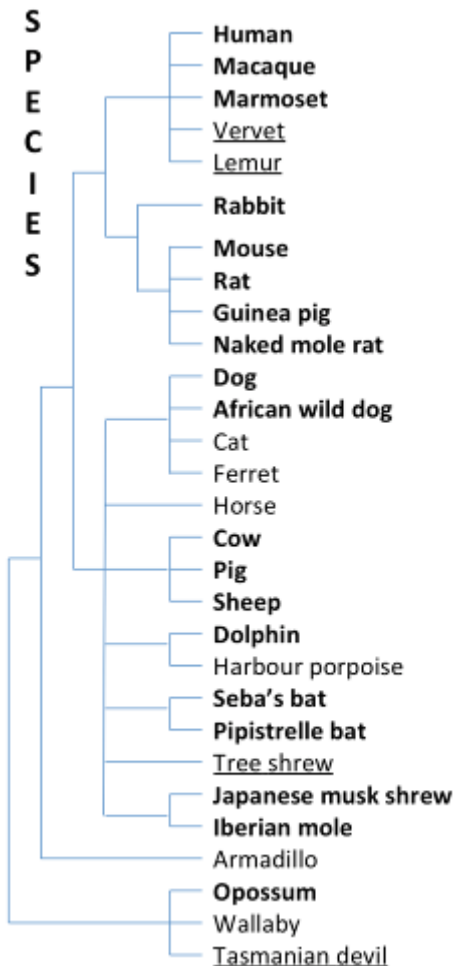
# High-throughput sequencing and comparative genomics in mammalian liver tissue



# Multiple intra- and inter-lineage evolutionary comparisons will further the understanding of regulatory genome evolution in mammals



**AIM:** Obtain post-mortem tissue samples for a representative cross-section of mammalian species



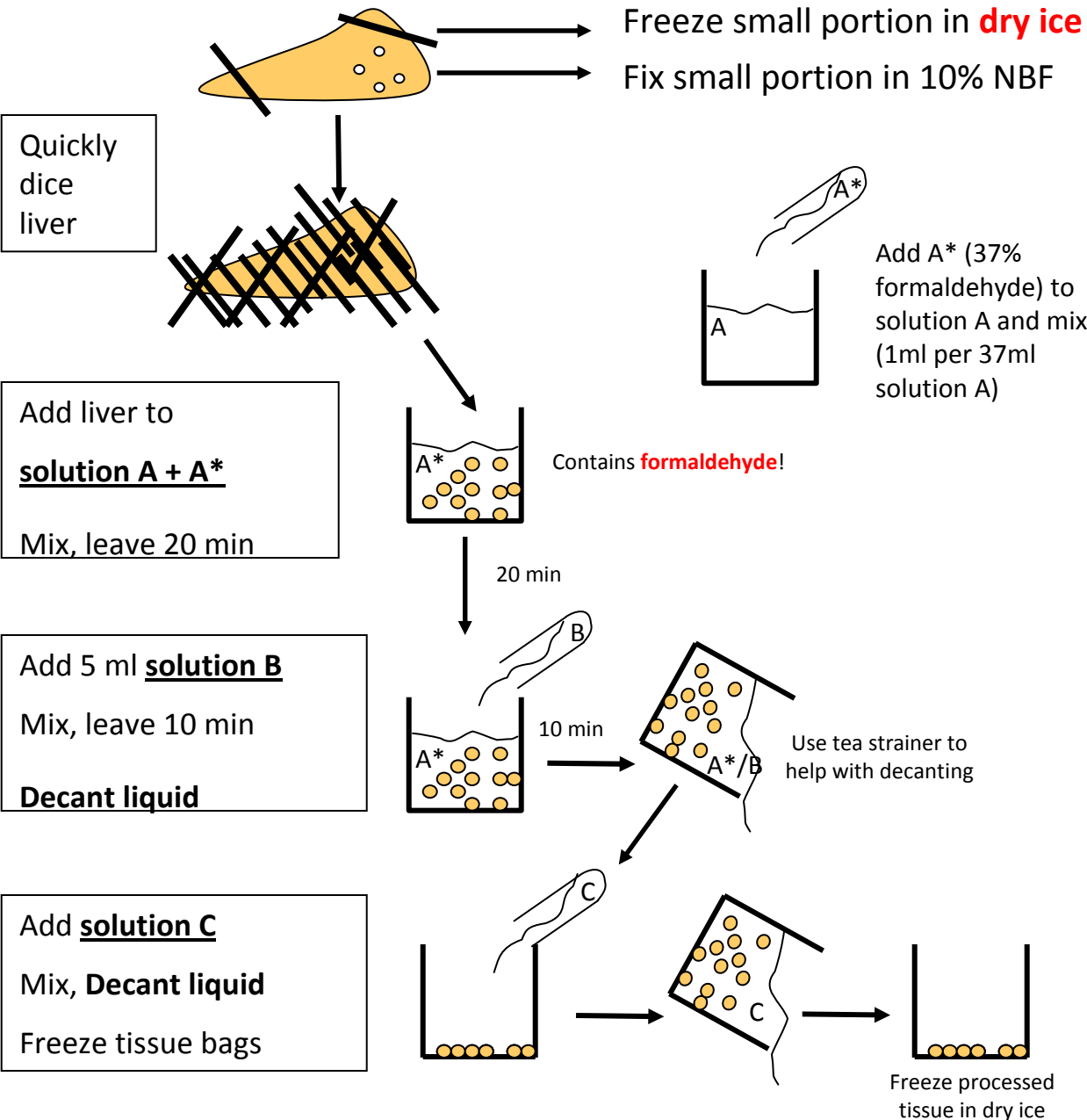
- ◆ Research colonies in external animal facilities
- ◆ Abattoirs/slaughterhouses
- ◆ Zoos
- ◆ Voluntary animal carers
- ◆ Collaboration with order-specific research programmes
- ◆ Field work



Health and biosafety challenges



# Overview of post-mortem tissue harvesting procedure



- 1) Dissect out liver onto kim-wipe moistened with PBS (solution C).
- 2) Perfuse liver with PBS to remove blood
- 3) Snap freeze 2-4 pieces of liver (approx 0.5cm<sup>2</sup>) in cryo tubes.
- 4) Fix 1-2 pieces liver in 10% NBF (60 ml tubes) (approx 0.5 - 1.0 cm<sup>2</sup>)

## 5) X-link procedure (A, B, C)

Before beginning add A\* to Solution A (A\*; contains formaldehyde).

(A): dice liver on kim-wipe moistened with PBS (solution C). Add liver to solution A + A\*. Mix and leave 20 min.

(B) After 20 minutes add approx 1/10 volume of solution B to solution A to quench formaldehyde. Mix and leave 10 minutes.

(C) Decant liquid and fill vial with solution C (PBS rinse; use tea strainer to help with decanting). Mix and decant liquid. Freeze liver in the same tube.

## Minimizing risk during sample collection

- a) **Transport of contaminant/hazardous chemicals and biological material**
- b) **Sample collection in external research facilities**
- c) **Sample collection in non-research facilities**
- d) **Sample collection in the field**
- e) **Storage and handling of harvested tissues at CRI**

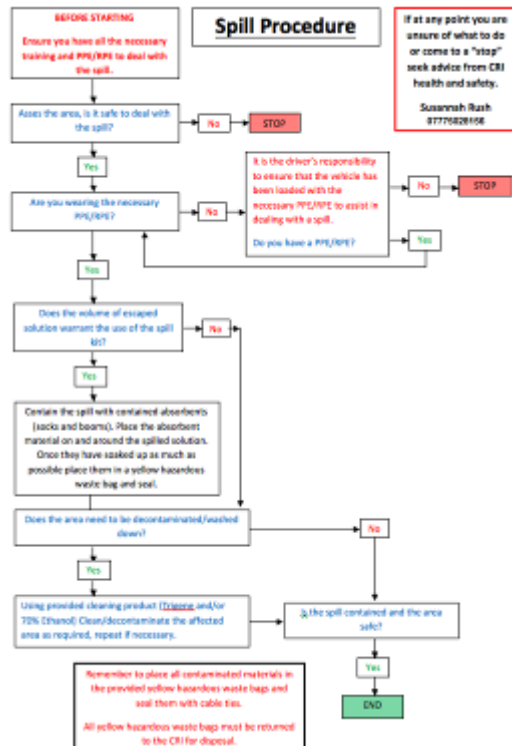


# Transport of chemicals and biological material



Carried during transport:

- Emergency procedures
- Safety equipment (goggles, spill kit, protective clothing, etc)
- Samples and chemicals in sealed containers



## Sample collection: external research facilities

- \* Prior consultation on site-specific risk assessments / adapt to local protocols
  
- \* Safety measures to implement at external facilities:
  - Ensure adequate ventilation
  - Respiratory protection if needed
  - Chemical/biological waste disposal
  - Decontamination

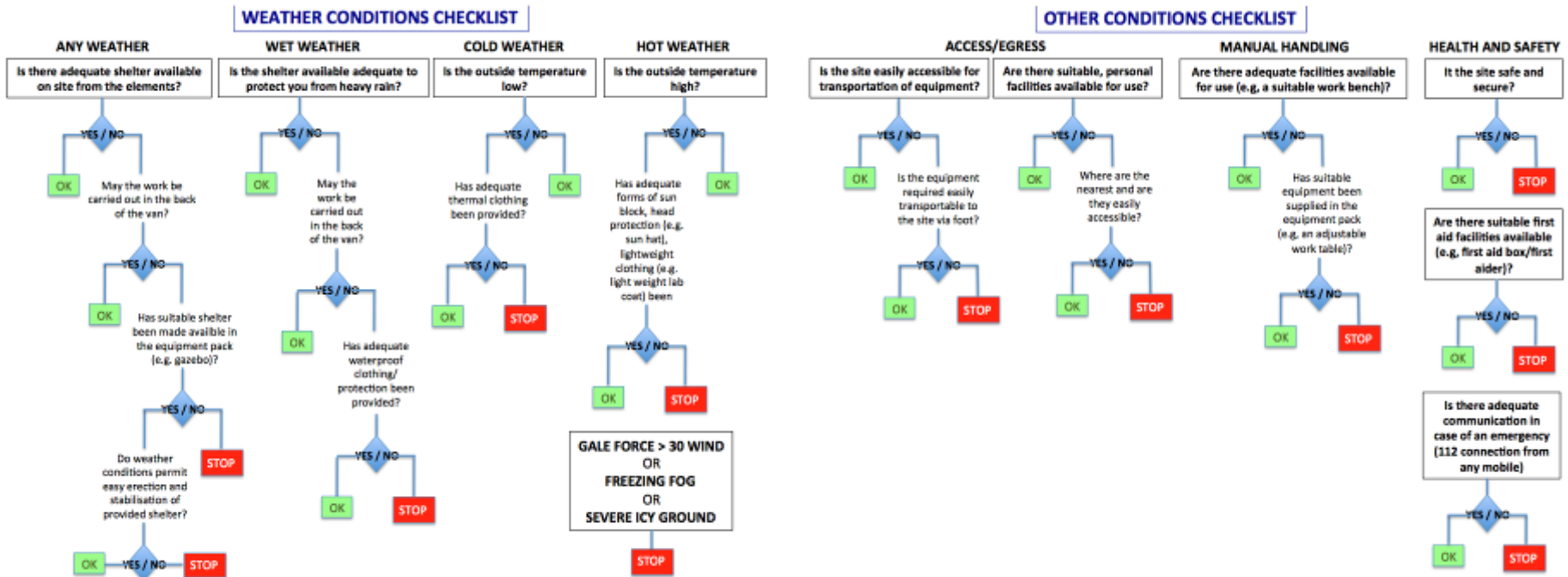
## Sample collection: non-research facilities

- \* Consultation on site-specific risks (e.g. abattoirs and laboratory work separated from animals for human consumption)
- \* Adapt procedure to local infrastructure:
  - Ensure adequate ventilation
  - Minimize contamination risk
  - Thorough decontamination after collection



# Sample collection in the field

- Assess adequate work conditions (e.g. weather, emergency contact)



- Portable and wireless equipment

## Storage and handling of harvested tissues

- \* Most tissues are screened for known disease (e.g. abattoirs and zoos), but zoonosis hazard is still present
  
- \* Biological contamination risk is minimized by several measures:
  - Bulk of tissue is fixed on collection (1% formaldehyde or 10% NBF)
  - Harvested tissues are stored in dedicated freezers
  - Tissues are manipulated in safety ventilated cabinets outside tissue culture rooms

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