TP53

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Background

Understanding TP53

Tumor Suppressor Function

p53 protein role in DNA repair, cell cycle regulation.

Importance in Cancer Studies

Mutations linked to various cancers; potential targets for gene therapy.

Cross-Species Perspective

TP53 found across mammals, amphibians, and more. Potential insights from cancer-resistant species.

Established Research

Studies show controlled p53 regulation vital for regeneration

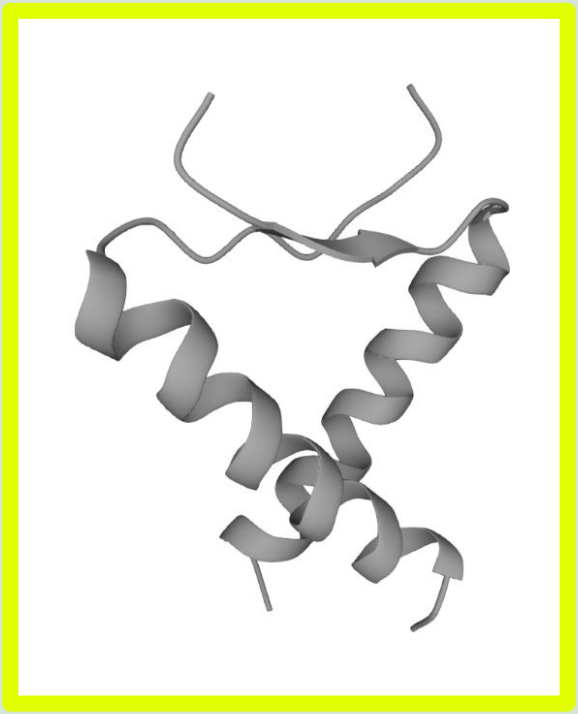
Our Approach

Gather TP53 sequences, align them, construct phylogenetic trees to reveal evolutionary patterns.

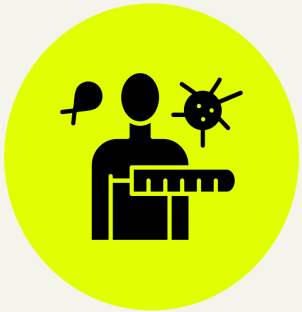
What is TP53?

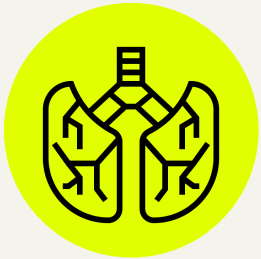
Understanding it's function

- Often referred to as: "Guardian of the genome".
- Gene function: detects damaged DNA, activates DNA repair mechanisms, and triggers cell cycle apoptosis if necessary.
- Its role helps suppress the formation of cancerous tumors.



Importance in Cancer Research





TP53 is susceptible to mutations, linked to various cancers (breast, lung, bladder) and syndromes (Li-Fraumeni)

Why understanding TP53 matters: Improves gene therapy and cancer treatments

Cross-Species Perspective

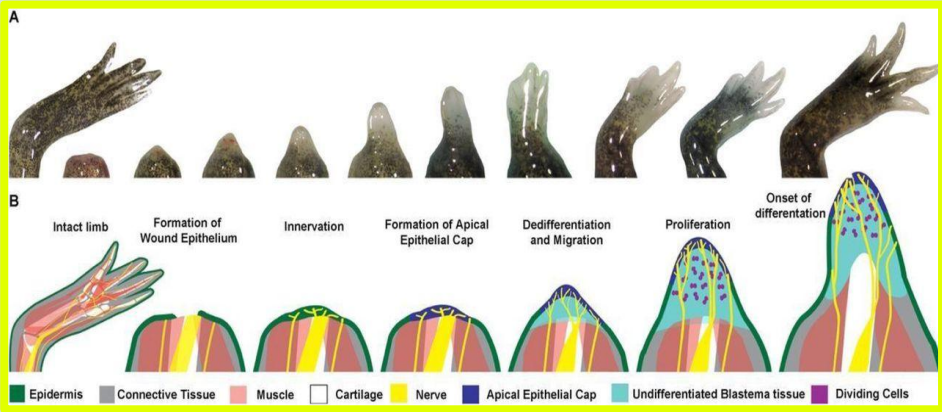
Ambystoma Mexicanum

Loxodonta Africana

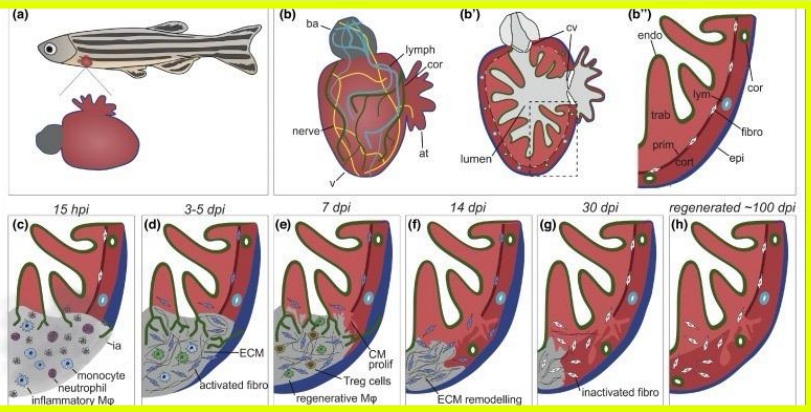
Podarcis muralis

- tp53 is not unique to humans: found in axolotls, lizards, elephants, chimpanzees etc.
- Some species show enhanced cancer resistance or regenerative capabilities
- Comparing sequences may reveal evolutionary clues to cancer resistance

Prior Research



Ambystoma Mexicanum



Danio Rerio

- A study found that controlled regulation of p53 is essential for the regeneration of limbs in salamander and fish species.
- The activity of tp53 initially decreases and then returns to baseline throughout each phase.
- Axolotl TP53 differs only by 38 amino acids; potential CRISPR applications.

Our Plan

Research questions: What increases cancer resistance?

- Is age/evolutionary stress a factor?
- Are there unique TP53 gene patterns that correlate with cancer resistance or regenerative traits?
- Does this gene vary across animal classes?
- Do similar sequences mean similar resistances?

Expected findings

- We expect to see clustering between animals within the same category.
- Clustering between animals with shared traits.

The Process

- Gather sequences of the TP53 gene of different animals.
- Align these sequences and create a phylogenetic tree, using software.
- Ascertain the evolutionary relationships of tp53 sequences.

Hypothesis

Animals with more developed/complex TP53 genes are able to counteract the susceptibility to diseases, leading to increased cancer resistance.

Data Collection

Gathering high-quality genetic data

Data Source: NCBI GenBank

- **Mammalian Sequences:** Includes various primates, horses, whales, naked mole rats, bats, etc.
- **Non-Mammalian Sequences:** Includes axolotls, salamanders, birds, fish, and reptiles.
- All sequences saved in FASTA format for compatibility with alignment tools.

Special Considerations

- Tumor Protein P53 Ortholog Database
- Primary source for wild-type TP53 sequences across various taxa.
- **Disease-Associated Sequences:** Horse TP53 (SSC) & Woodchuck TP53 (Hepatitis Virus):

Data Retrieval and Organization

- Focused on coding regions to highlight functional differences.
- Disease-associated sequences provide insights into TP53 mutations and their broader impacts.

Alignment Method using python

Needleman Wunsch Algorithm Implementation

- **Alignment Matrix Construction:** The algorithm computes a two-dimensional scoring matrix. Scores are based on the match (+1), mismatch (-1), and gap penalties (-2) defined in our scoring system.

- **Traceback:** After building the alignment matrix, the code traces back through the matrix to find the optimal alignment path.This ensures that the best possible alignment is generated for the sequences.

  Motif Sequence Calculation: A consensus or motif sequence is identified by analyzing aligned regions, highlighting conserved amino acids that are functionally significant.

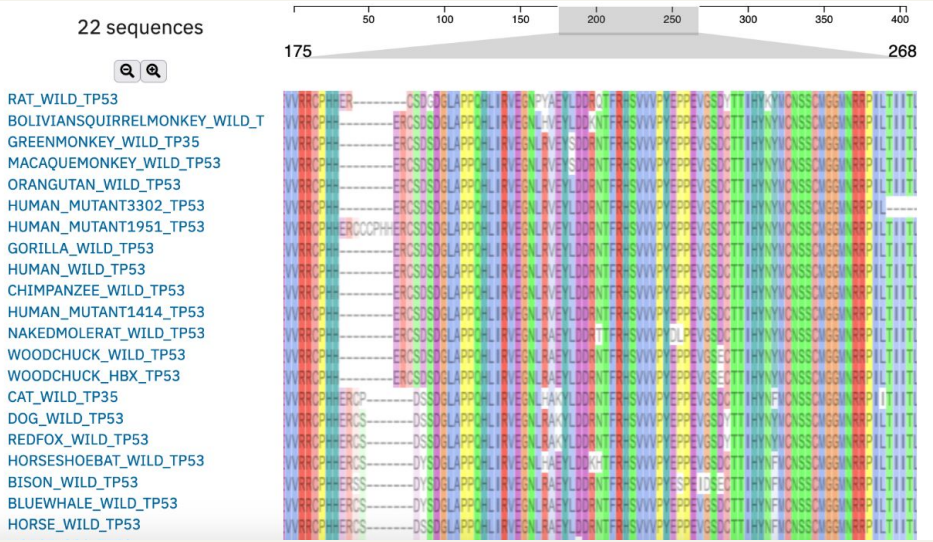
- **Gapping Function:** Code refines alignments by removing unnecessary gaps, making the results more readable.

```
#STEP 3: FILL IN THE MATRIX.
match = 1
mismatch = -1
gap = -2
for i in range(1, s1Length +1):
    for j in range(1, s2Length + 1):
        deletion = Matrix2D[i -1][j] +gap  # Deletion (gap in seq2)
        insertion = Matrix2D[i][j -1] +gap  # Insertion (gap in seq1)
        matchScore = Matrix2D[i -1][j -1] + (match if seq1[i-1] == seq2[j-1] else mismatch)
        Matrix2D[i][j] = max(deletion, matchScore,insertion)  # Pick the best score

# STEP 4: TRACEBACK
align1 = []
align2 = []
i = s1Length
j = s2Length

while j and i > 0:
    currScore = Matrix2D[i][j]
    # statement handles match and mismatch case
    if Matrix2D[i - 1][j - 1] + (match if seq1[i - 1] == seq2[j - 1] else mismatch)==currScore:
        align1.append(seq1[i - 1])
        align2.append(seq2[j - 1])
        j=j-1
        i=i-1
    # gap in seq2
    elif  Matrix2D[i - 1][j] + gap==currScore:
        align2.append("-")
        align1.append(seq1[i - 1])
        i=i-1
    # gap in seq1
    else:
        align2.append(seq2[j-1])
        align1.append("-")

        j=j-1
# add all remaining gaps if ran out of characters in one sequence
while i > 0:
    align2.append("-")
    align1.append(seq1[i - 1])
    i = i-1
while j > 0:
    align1.append("-")
    align2.append(seq2[j - 1])
    j=j-1
# reverse the sequences to return in the correct order
align1 = ''.join(reversed(align1))
align2 = ''.join(reversed(align2))
return align1, align2
```

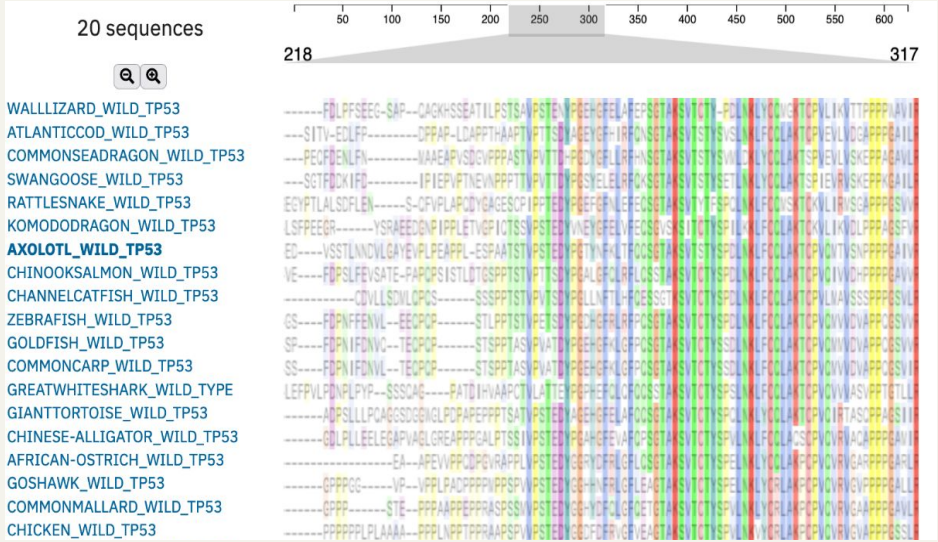
Alignment Interpretations

```
Seq1    PPPGTRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSV
Seq2    PPPGTRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSV
Seq3    PPPGTRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSV
Seq4    PPPGTRVRAMAIYKQSQHMTEVVRRCP-HHERCCCPHHERCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSV
Seq5    PPPGTRVRAMAIYKKSEFMTEVVRRCP-HHER----------CSDSSDGLAPPQHLIRVEGNLRAEYLDDRNTFRHS
Seq6    PPPGTRVRAMAIYKKSEFMTEVVRRCP-HHER----------CSDSSDGLAPPQHLIRVEGNLRAEYLEDRNTFRHS
Seq7    TPPPGTRVRAMAIYKKSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRAEYLDDRNTFRHS
Seq8    TPPPGTRVRAMAIYKKSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRAEYLDDRNTFRHS
Seq9    PPPGSRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRVEYSDDRNTFRHSV
Seq10   PPPNTCVRAMAIYKKSEFVTEVVRRCP-HHER----------CSDSSDGLAPPQHLIRVEGNLRAKYLDDRNTFRHS
Seq11   PPPGTCVRAMAIYKKSEFMTEVVRRCP-HHER--------CPDS-SDGLAPPQHLIRVEGNLHAKYLDDRNTFRHSV
Seq12   TSTPPPGTRVRAMAIYKKSQHMTEVVRRCP-HHER----------CSDGDGLAPPQHLIRVEGNPYAEYLDDRQTFR
Seq13   PPPGSRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRVEYSDDRNTFRHSV
Seq14   PPRGTRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLHVEYLDDKNTFRHSV
Seq15   PPPGTRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSV
Seq16   PPPGTRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSV
Seq17   PPPGTRVRAMAIYKQSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSV
Seq18   PPPVGTRVRAMAIYKKSEYMTEVVRRCP-HHER----------CSDYSDGLAPPQHLIRVEGNLHAEYLDDKHTFRH
Seq19   PPPGTRVRAMAIYKKLEHMTEVVRRCP-HHER----------SSDYSDGLAPPQHLIRVEGNLRAEYLDDRNTFRHS
Seq20   PPPNTCVRAMAIYKKSEFVTEVVRRCP-HHER----------CSDSSDGLAPPQHLIRVEGNLRAKYLDDRNTFRHS
Seq21   PPPPGTRVRAMAIYKKSQHMTEVVRRCP-HHER----------CSDSDGLAPPQHLIRVEGNLRAEYLDDRTTFRHS
Seq22   PPPGTRVRAMAIYKKSEYMTEVVRRCP-HHER----------CSDYSDGLAPPQHLIRVEGNLRAEYLDDRNTFRHS
```

Mammalian Sequences



Non-mammalian Sequences



Phylogenetic Analysis

Multiple Sequence Alignments

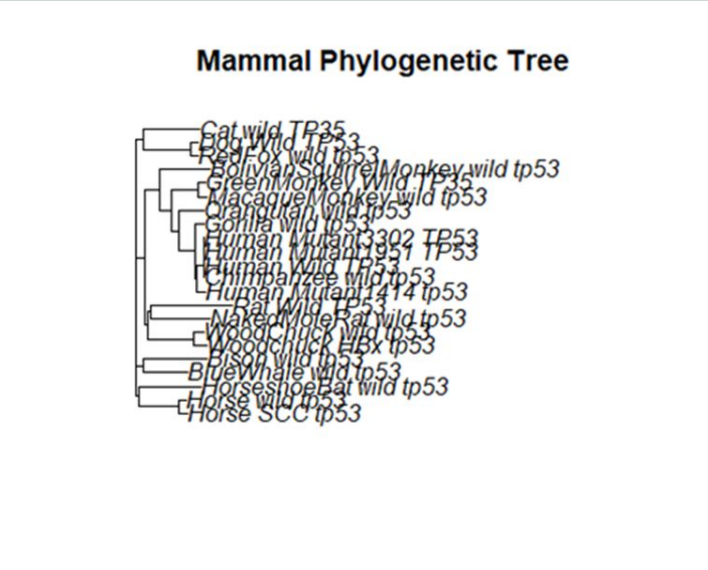
Mammals
Produced through R tools

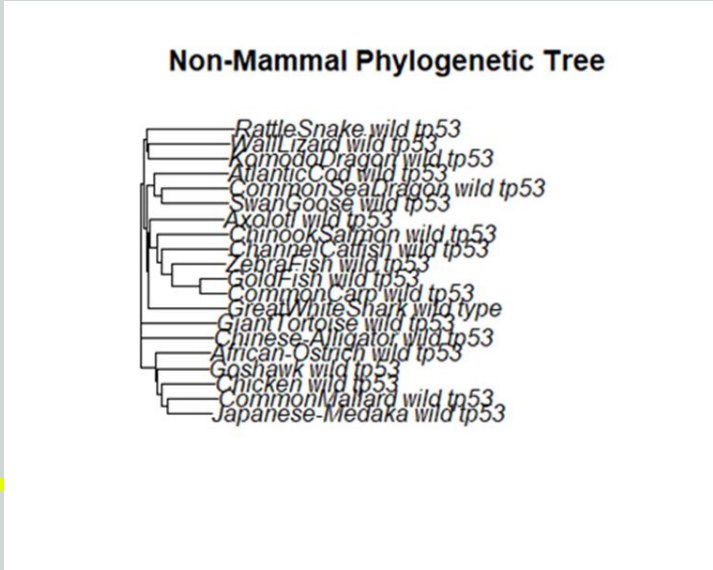
Non-mammals
Produced though R tools

Union
Produced through Phylogeny.fr

Phylogenetic Trees

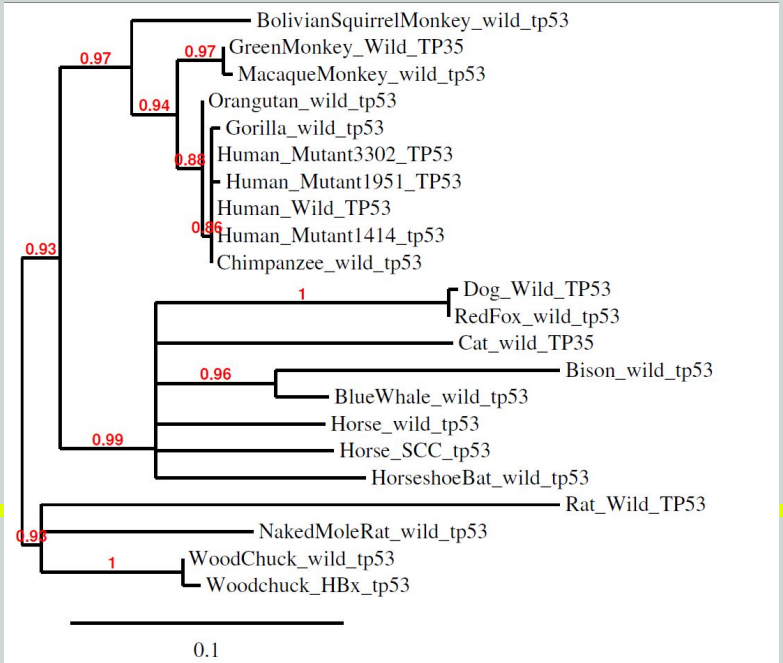
Construction with R tools

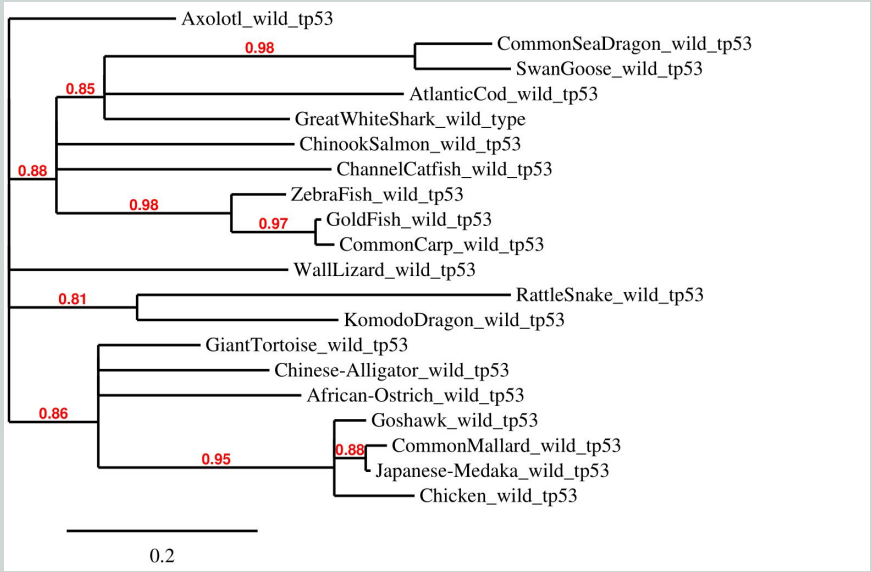




Phylogenetic Trees

Validation with Phylogeny.fr





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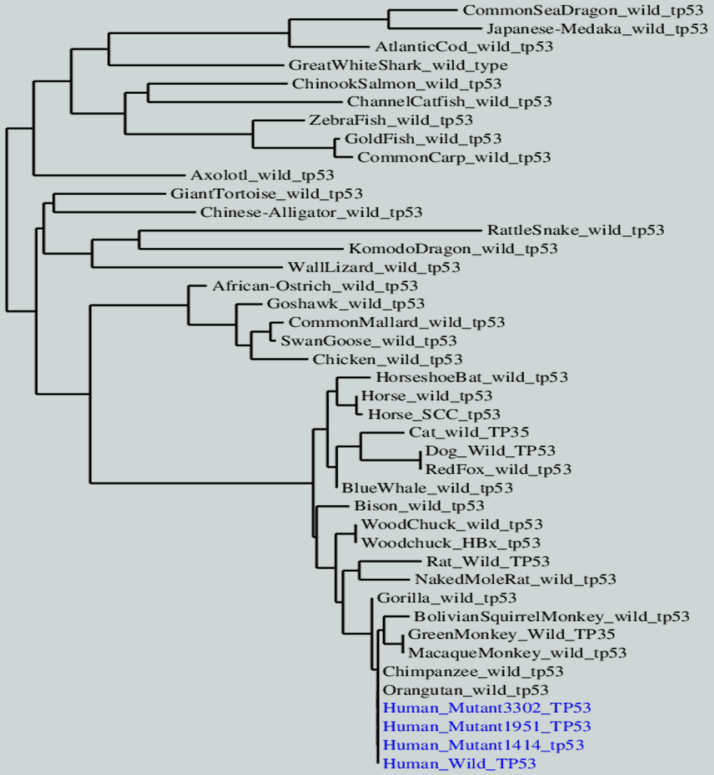

Findings

Taxons do not coincide with traits

Many of the taxons of the trees reveal evolutionary relationships between who do not exhibit similar cancer resistance traits
(ie. Blue Whale, Bison, and Swan Goose, Mallard)

Furthermore, animals who share cancer resistant traits like the mole rat and axolotl are far removed from one another suggesting multiple types of cancer resistance methods among genes

Axolotl's unique TP53 gene

Axolotls, whose TP53 gene usage is linked to their regenerative and cancer resistance ability, seem to unique and appear as an outgroup in both trees

Limitations and Improvements

MSA Improvements:

- Some sequences in the alignment appear to have excessive or misaligned gaps. Reviewing how the gap penalties are applied in the scoring matrix, could improve accuracy.
- Potential issues in matrix initialization: If sequences are not aligning correctly at the ends or mid-regions, the problem could lie in the initial matrix setup.
- Code was very slow, taking upwards of 40 seconds to output an alignment.

Limitations:

- Linking phylogenetic analysis to traits was cloudy at best given the multiple confounding factors between evolutionary relationships and cancer resistance traits
    - Analysis of the correlation between life expectancy, evolutionary stress and cancer resistance was further stunted by this limitation

Future Work

Key Findings / Limitations	Recommendations
01 Taxons don’t coincide with traits	Long Term Study of the development of certain cancers in certain environments for species with observed traits to determine how each gene deals with cancer resistance uniquely
02 Confounding factors between evolutionary relationships and traits	
03 Axolotl’s Unique Gene	Study comparing Axolotl’s TP53 gene and upkeep to other animals with observed resistances

Thank You!

Thank you for listening! Questions?