Studiul genei *CG18135* de la *Drosophila melanogaster* prin utilizarea unor metode avansate de Analiză Genetică, Genomică și Bioinformatică

- rezultate preliminare -

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Categoria: Științe biologice

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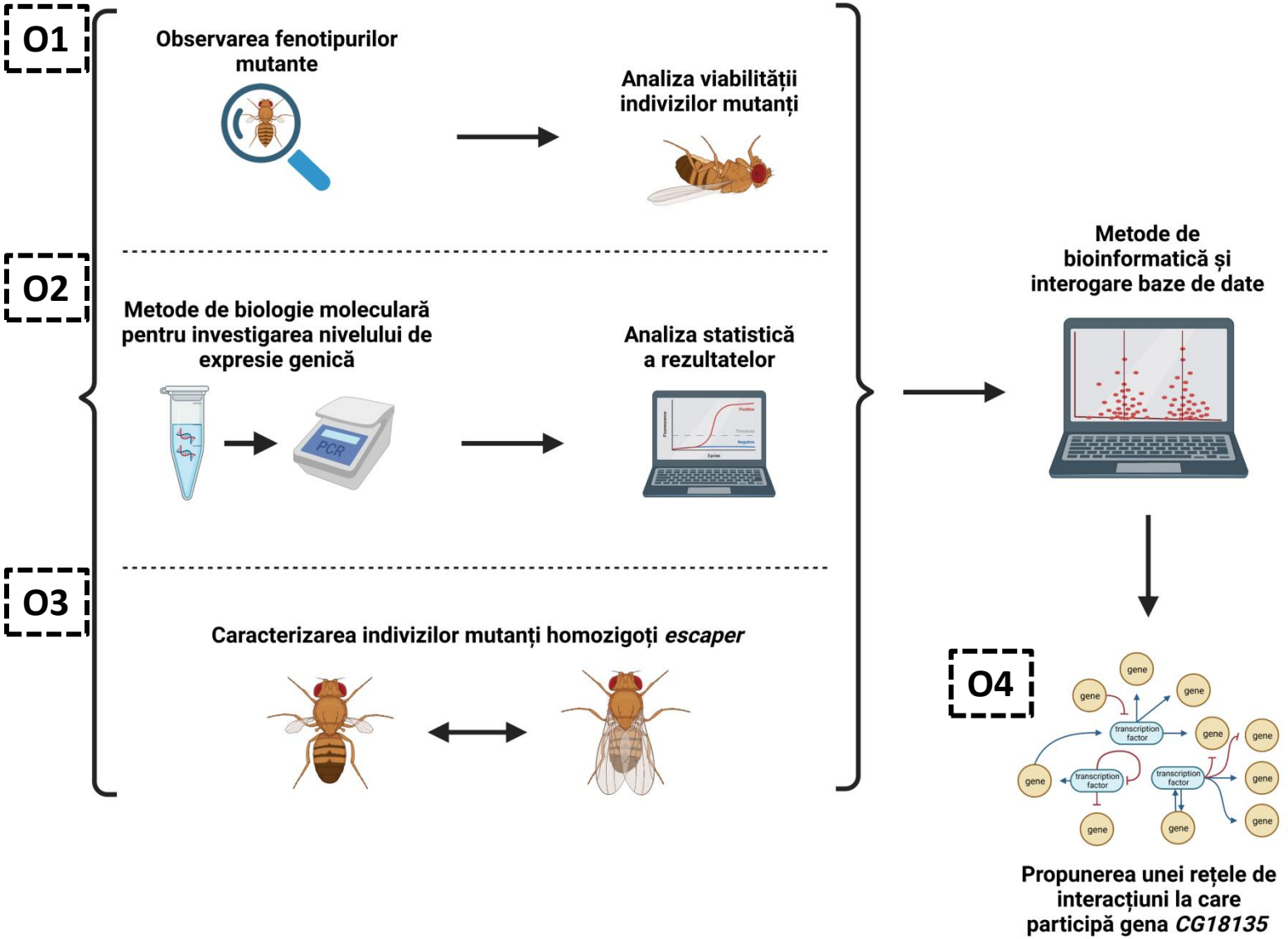
Scop și obiective



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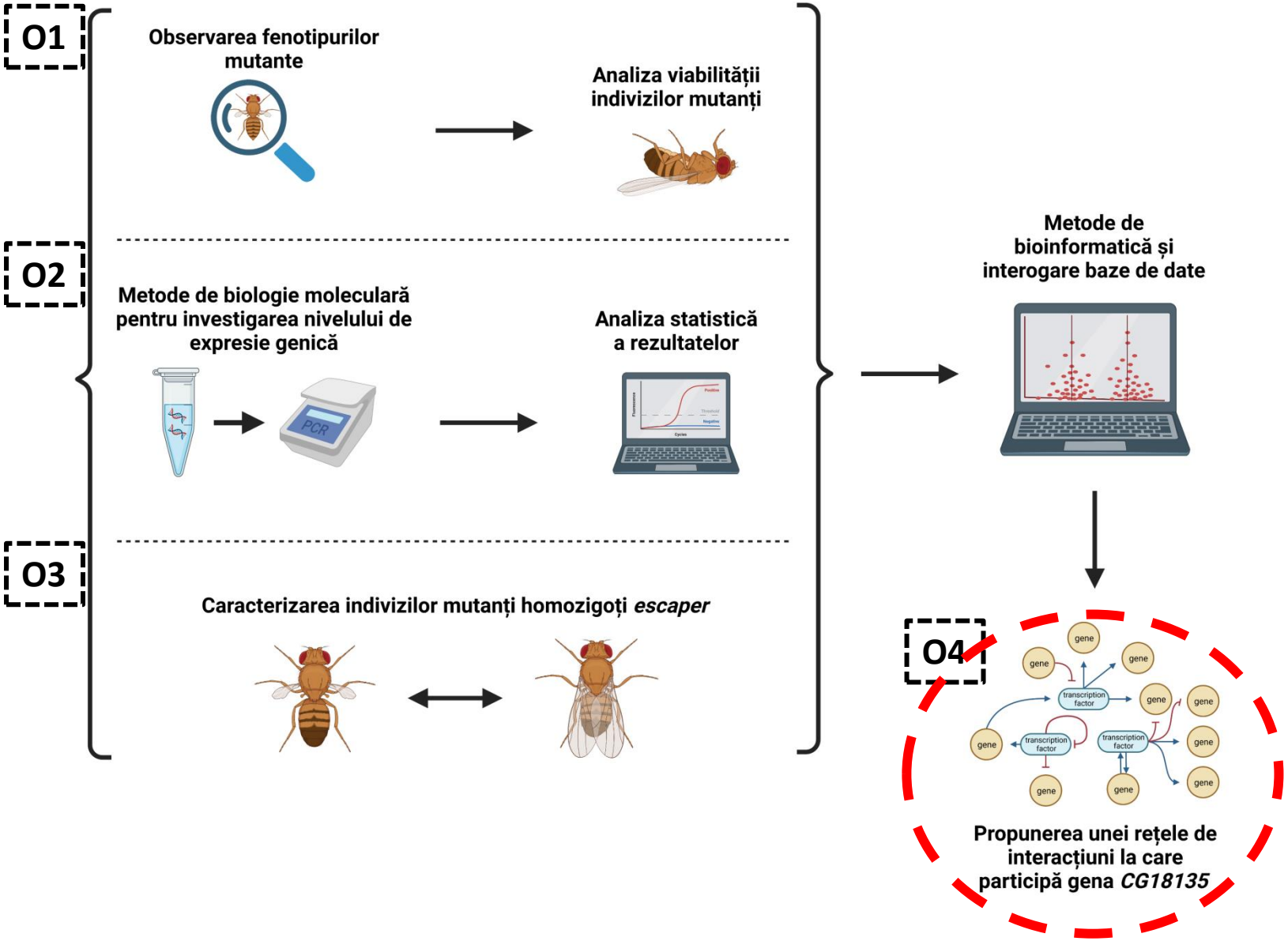

Publicații

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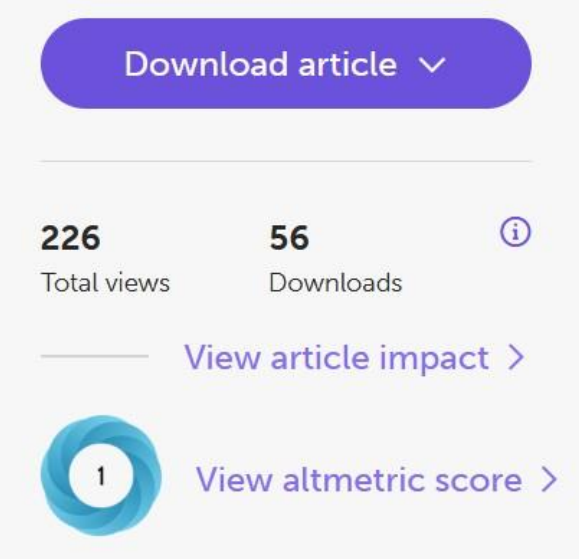
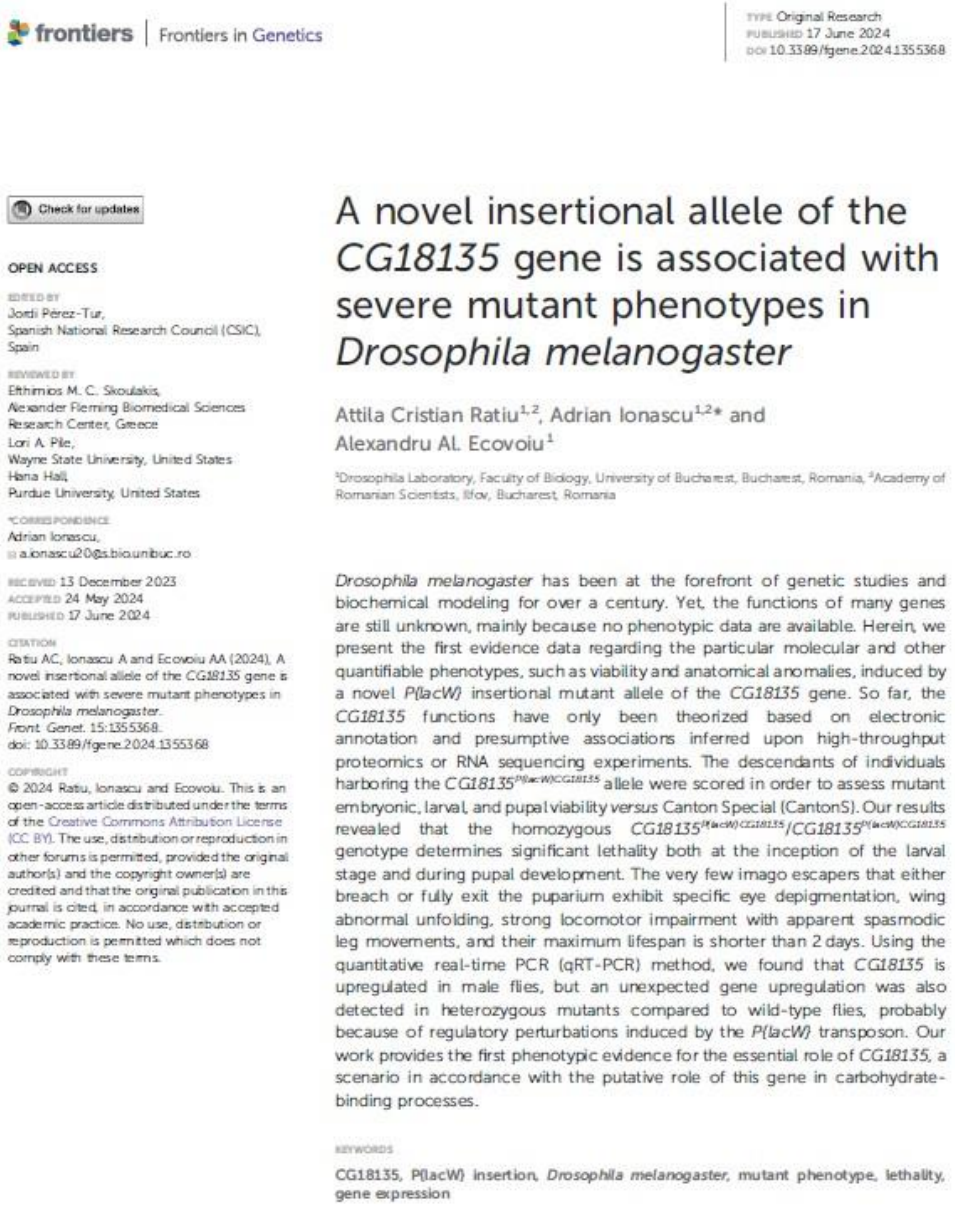
- AIS = 0.937 (Q2)
- Factor de impact = 2.8





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A novel insertional allele of the *CG18135* gene is associated with severe mutant phenotypes in *Drosophila melanogaster*

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Drosophila melanogaster has been at the forefront of genetic studies and biochemical modeling for over a century. Yet, the functions of many genes are still unknown, mainly because no phenotypic data are available. Herein, we present the first evidence data regarding the particular molecular and other quantifiable phenotypes, such as viability and anatomical anomalies, induced by a novel *P{lacW}* insertional mutant allele of the *CG18135* gene. So far, the *CG18135* functions have only been theorized based on electronic annotation and presumptive associations inferred upon high-throughput proteomics or RNA sequencing experiments. The descendants of individuals harboring the $CG18135^{P\{lacW\}CG18135}$ allele were scored in order to assess mutant embryonic, larval, and pupal viability *versus* Canton Special (CantonS). Our results revealed that the homozygous $CG18135^{P\{lacW\}CG18135}/CG18135^{P\{lacW\}CG18135}$ genotype determines significant lethality both at the inception of the larval stage and during pupal development. The very few imago escapers that either breach or fully exit the puparium exhibit specific eye depigmentation, wing abnormal unfolding, strong locomotor impairment with apparent spasmodic leg movements, and their maximum lifespan is shorter than 2 days. Using the quantitative real-time PCR (qRT-PCR) method, we found that *CG18135* is upregulated in male flies, but an unexpected gene upregulation was also detected in heterozygous mutants compared to wild-type flies, probably because of regulatory perturbations induced by the *P{lacW}* transposon. Our work provides the first phenotypic evidence for the essential role of *CG18135*, a scenario in accordance with the putative role of this gene in carbohydrate-binding processes.

KEYWORDS
CG18135, P{lacW} insertion, *Drosophila melanogaster*, mutant phenotype, lethality, gene expression

Contextul genomic

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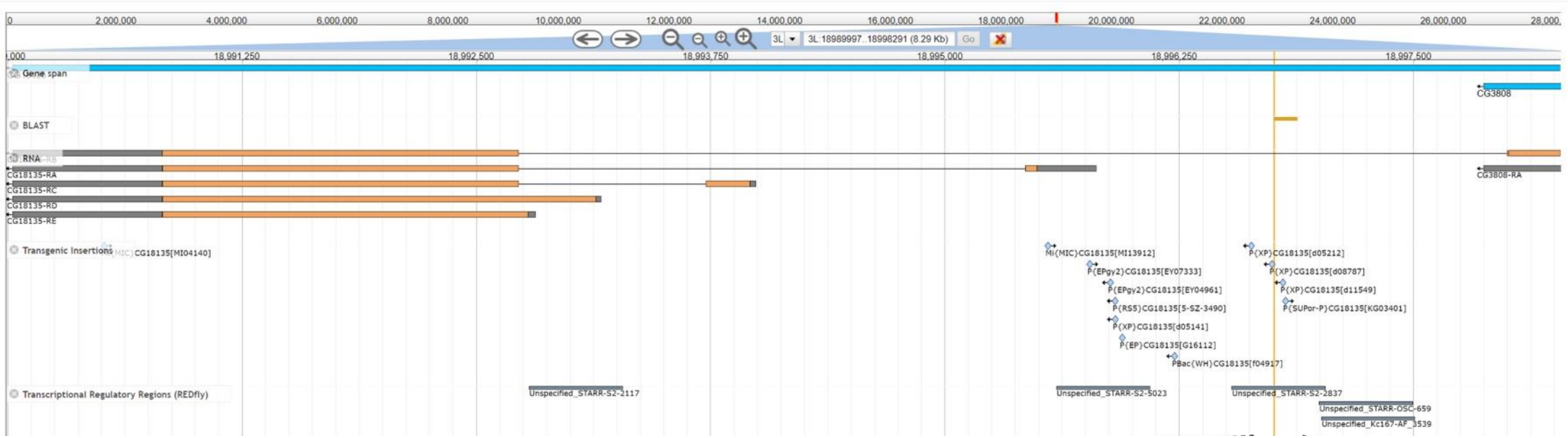
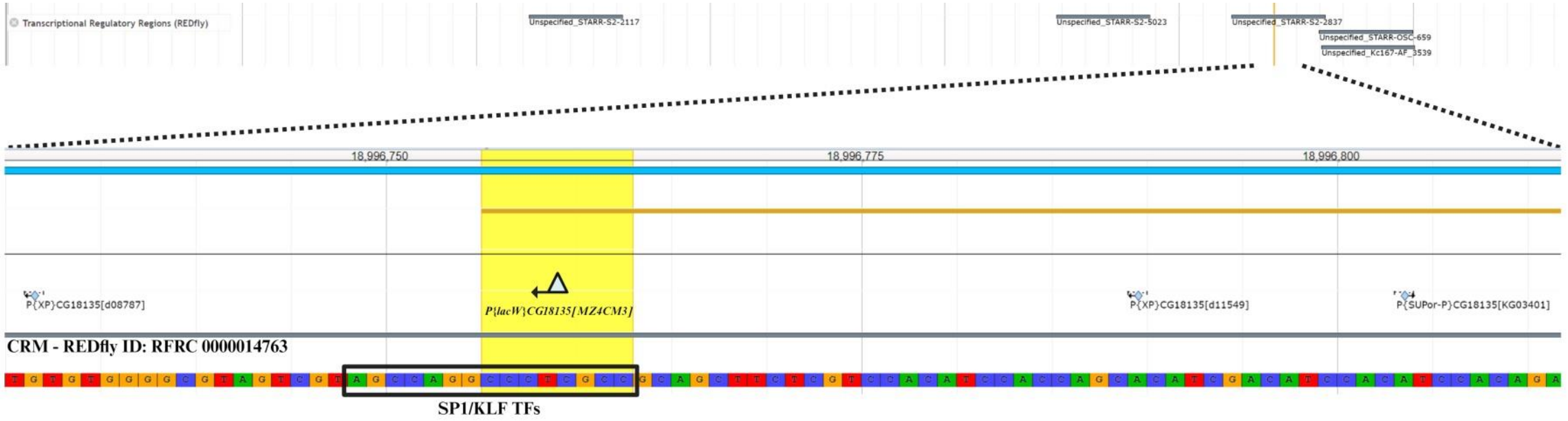


- Inserție *P{lacW}* la coordonatele genomice 3L:18996755-18996762
- Un total de 12 inserții ale transpozonilor derivați din *P*-, *PBac*- și *MiMIC*- au fost raportate pentru gena *CG18135*
- Inserția $P\{lacW\}^{CG18135.MZ4CM3}$ și alte patru inserții ale transpozonilor derivați din *P*- sunt suprapuse sau în apropierea modului reglator în cis CRM14763

Contextul genomic

- Analiza XSTREME (MEME Suite) a indicat prezența unor situsuri de legare pentru factorii de transcriere SP1/KLF

- Motivul de legare al factorilor de transcriere SP1/KLF are secvența de nucleotide AGCCAGGCCCTCGCC și conține secvența țintă a transpozonului (subsecvența CCCTCGCC)



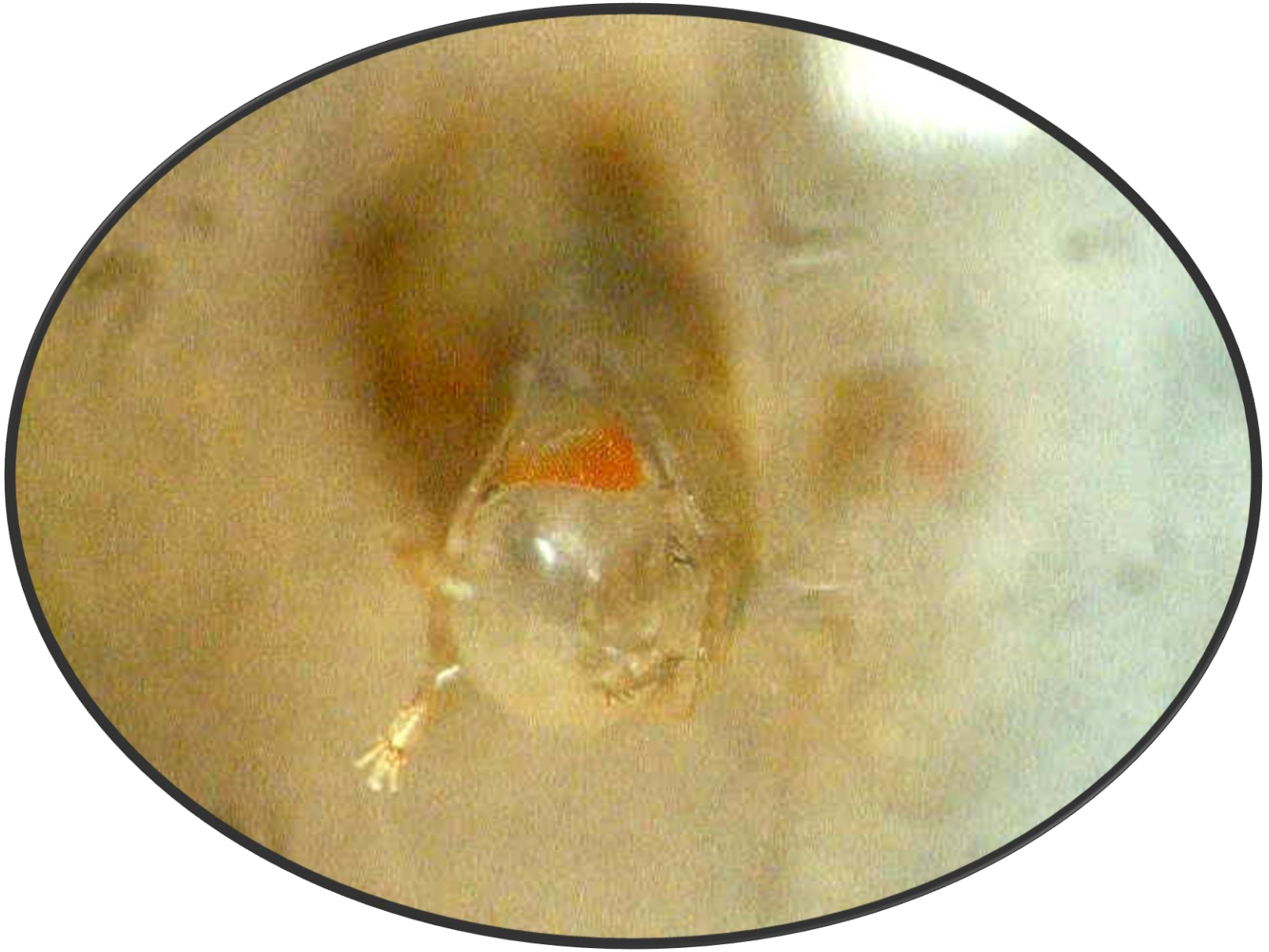
Context genomic

- Factori de transcriere din SP1/KLF precum Sp1, buttonhead și cabut (cbt) au rol de activatori sau represori în procese de dezvoltare

- Activarea anormală a cbt poate explica parțial:
  - fenotipul specific al aripilor observat în mutanții imago *escaper*
  - mecanismul de control al mușchilor implicați în zbor și locomoție
  - reglarea numărului de depresori dorsali ai mușchilor implicați în salt
  - comportamentul sedentar și mișcările spasmotice ale mutanților *escaper*

- Omologii *cbt* la om sunt *KLF10* și *KLF11* și sunt implicați în:
  - metabolismul energenic
  - proliferarea, diferențierea și apoptoza celulară mediată de TGF-β

Caracterizarea indivizilor *escaper* (raportare 2)



Caracterizarea indivizilor *escaper* (raportare 2)

Test de complementație genică

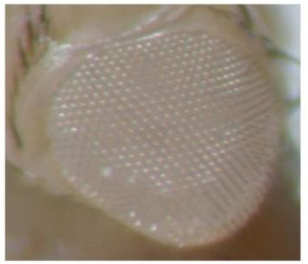
$$\frac{CG18135^{P\{lacW\}CG18135}}{\text{TM6B, } Tb, Hu, e} \times \frac{\gamma Cop^{P\{lacW\}057302}, CG18135^{P\{lacW\}CG18135}}{\text{TM6B, } Tb, Hu, e}$$

$$\frac{CG18135^{P\{lacW\}CG18135}}{\gamma Cop^{P\{lacW\}057302}, CG18135^{P\{lacW\}CG18135}}$$

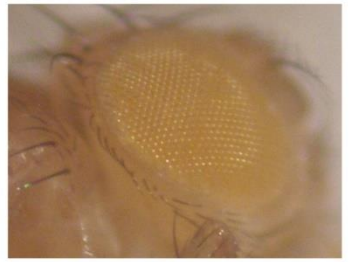
- infertili / sterili
- viabilitate crescută
- fenotipuri variate la nivelul aripilor și ochilor

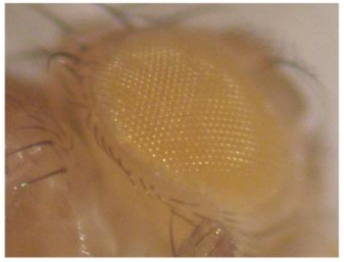
Mobilizarea inserției *P{lacW}CG18135.MZ4CM3*

$$\frac{CG18135^{P\{lacW\}CG18135}}{\text{TM6B, } Tb, Hu, e} \times \frac{\Delta 2\text{-}3, Sb}{\text{TM6B, } Tb, Hu, e}$$

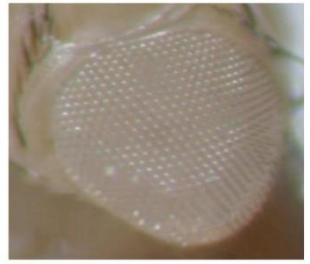


$$\frac{CG18135^{P\{lacW\}CG18135}}{\Delta 2\text{-}3, Sb} \times \frac{CG18135^{P\{lacW\}CG18135}}{\text{TM6B, } Tb, Hu, e}$$





$$\frac{CG18135^{\text{ excizie}}}{CG18135^{P\{lacW\}CG18135}} \text{ și } \frac{CG18135^{\text{ excizie}}}{\text{TM6B, } Tb, Hu, e}$$



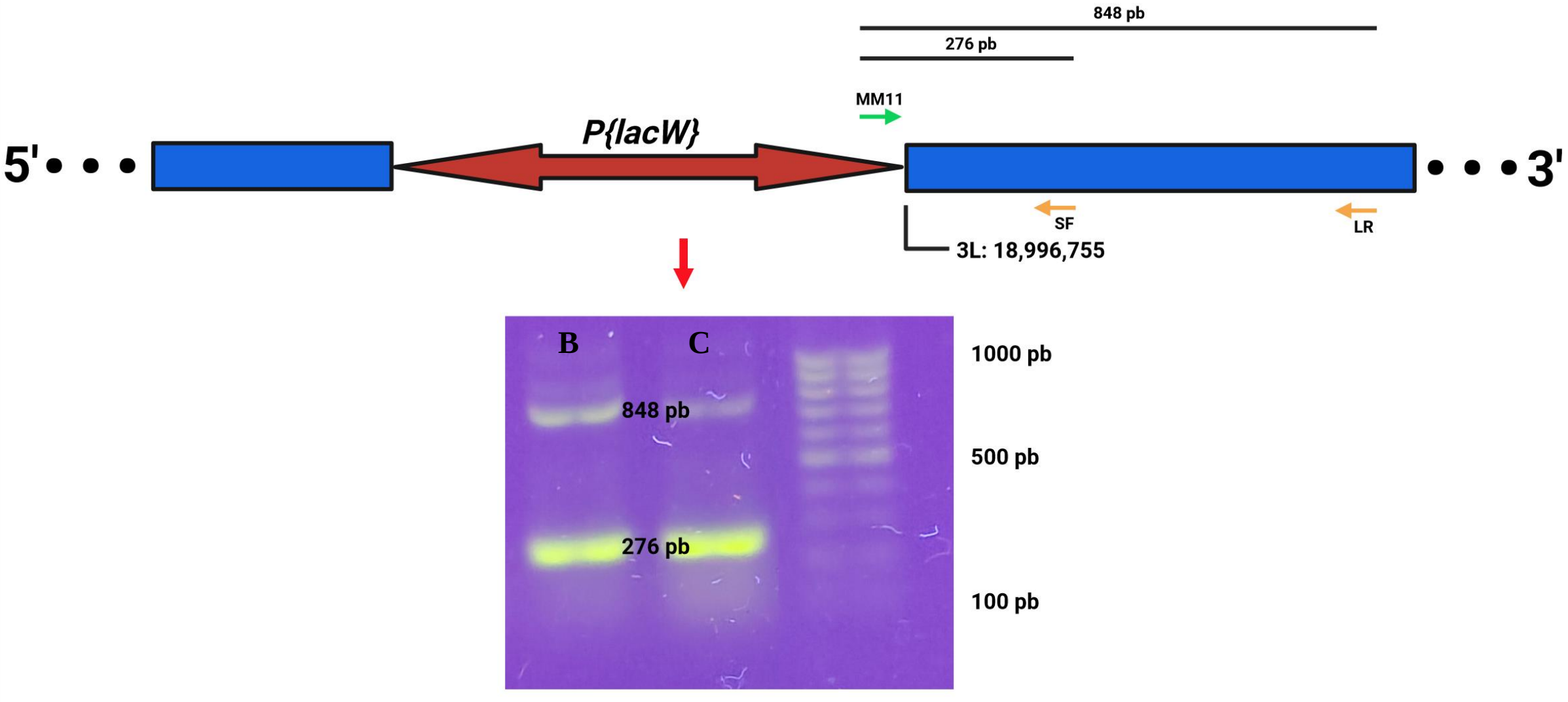
Evaluarea exciziei transpozonului prin PCR

- Indivizi selectați:
  - *CG18135*$^{P\{lacW\}CG18135}$?/ TM6B, *Tb*, *Hu*, *e* → **proba B**
  - *CG18135*$^{P\{lacW\}CG18135}$ */* TM6B, *Tb*, *Hu*, *e* → **proba C**

- Extracție ADN cu *kit*-ul Wizard Genomic DNA Purification kit (Promega)

- Amplificare multiplex cu:
  - 1 primer specific pentru capetele invers-repetate ale transpozonului *P{lacW}* → **MM11**
  - 2 primeri specifici pentru regiunea genomică din aval de inserție → **SF** și **LR**

Evaluarea exciziei transpozonului prin PCR



Concluzii

- Investigații *in silico* → *P{lacW}*$^{CG18135.MZ4CM3}$ poate produce o serie de perturbări funcționale la nivel genomic
- Teste de complementație → Fenotipul determinat de genotipul *CG18135*$^{P\{lacW\}CG18135}$/*CG18135*$^{P\{lacW\}CG18135}$ este influențat de structura *background*-ului genomic
- *Cross*-uri de reversie → Obținerea revertanților cu mutații de tip excizie de reversie care complementează alela *CG18135*$^{P\{lacW\}CG18135}$
- Testare PCR → Descendența din *cross*-ul de reversie conține doar inserția *P{lacW}* în gena *CG18135*

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Vă mulțumesc pentru atenție!