Identifying CKY5369 yeast cells with downstream shifts in TSS initiation to determine if there is a mutation in SSL2

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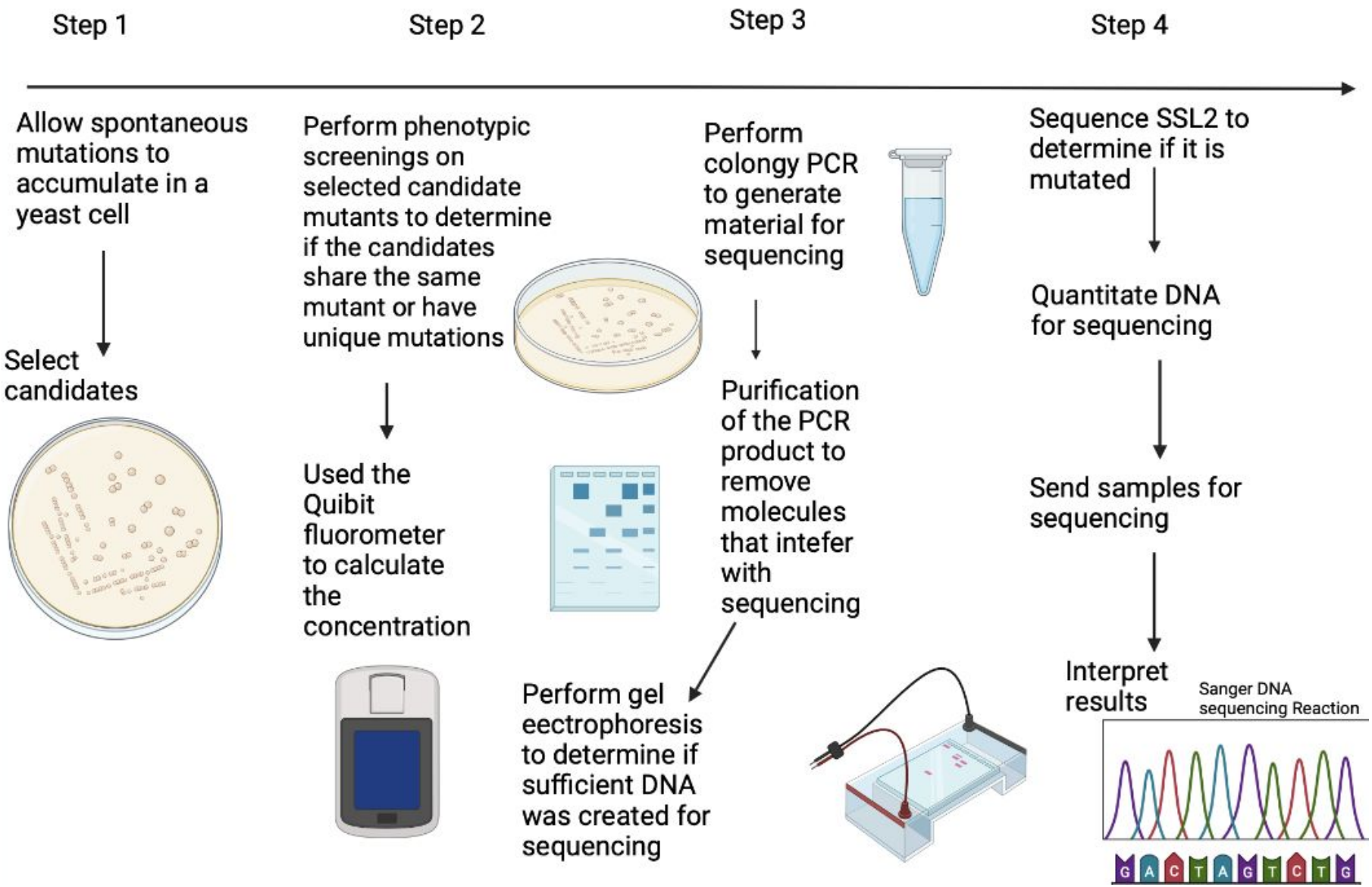
DNA Regulation and Diseases Foundations of Biology 2 Laboratory Course

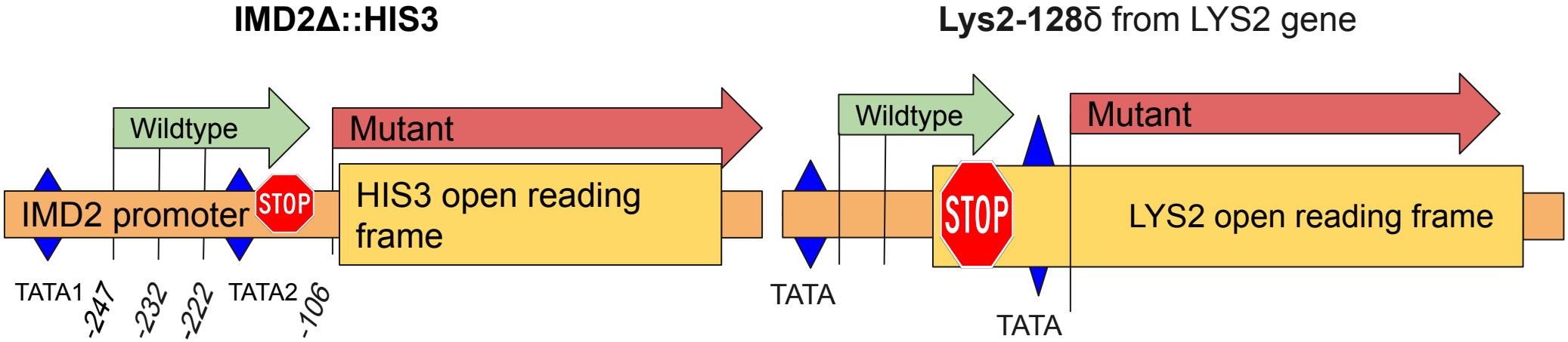
Extreme downstream shifts in transcription initiation can be caused by a mutation in the XPB/SSL2 subunit of TFIIH

- By studying mutations in the Ssl2 subunit of TFIIH, its role in transcription start site selection can be understood and applied to XPB mutations
  - xeroderma pigmentosum and trichothiodystrophy are diseases of XPB mutation
- TFIIH is a transcription factor with helicase and translocase activity
  - XPB is a subunit, homolog is Ssl2 in yeast
- Model organism: *Saccharomyces cerevisiae* (yeast) cells
  - Easily reproduced, easily modified auxotroph, small in size & contain similar DNA to humans
- Yeast mutant candidates will be grown on various controlled experiments with differences in media type & temperature → then sequenced to find similarities in mutations
  - Mutants will be selected under conditions that no growth is expected
  - Results will reveal reporter gene activation & effects of transcription termination

Experimental flowchart



Mutant CKY5369 alleles were selected from colonies grown on SC-H-K media

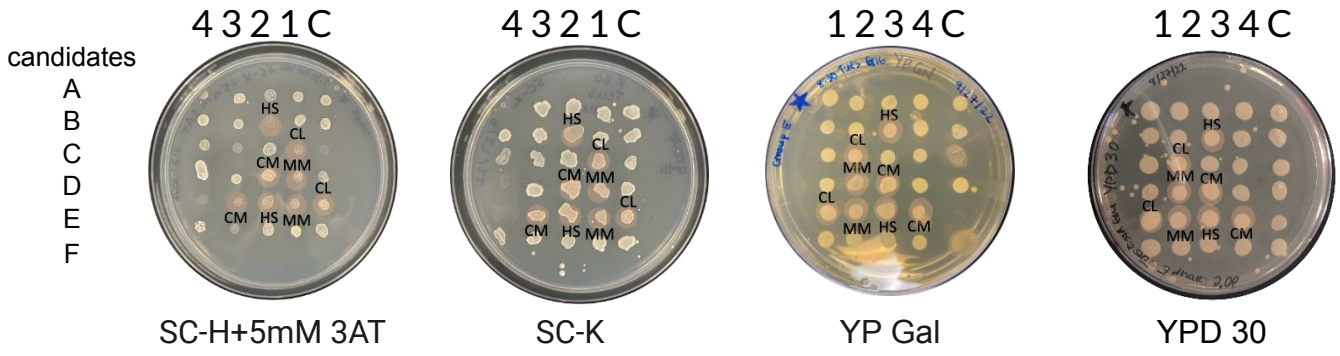


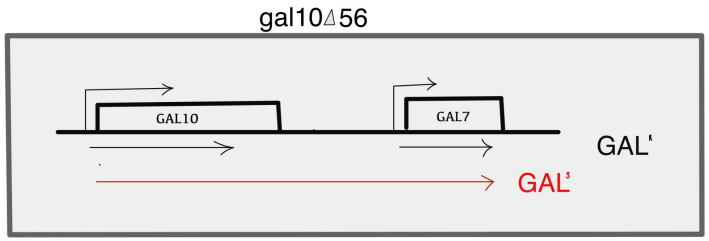
CKY5369 grown on SC-H-K for 7 days, incubated at 30℃

- In high GTP conditions, wild type cells do not grow on media lacking HIS3, the reporter HIS3 is not expressed
  - IMD2 gene was replaced by the HIS3 gene to identify growth on media lacking histidine
- Modifying the IMD2 gene allows us to distinguish down stream shifts in TSS
  - Wild type IMD2 gene would not make LYS2 if there were a downstream shift
- Mutants will make both HIS3 and LYS2 to be able to grow on media lacking both Histidine and Lysine

6 Chosen Candidates showing a phenotype pattern of Ssl2 with mutations with downstream shifts in TSS to continue analysis

- Favorable phenotypes: lack of growth on plates with galactose as well as growth on plates with raffinose, and those lacking histidine & lysine → no termination mutations
  - His+ → growth = strength of imd2Δ::HIS3 reporter activation with histidine nutrient inhibition
  - Lys+ → growth = strength of ys2-128δ reporter activation with lysine nutrient inhibition
- Ruling out possible termination mutations in candidates
  - Gal- → lack of growth = poor sugar source, strength of gal10Δ56 deletion preventing the GAL7 gene (--> no growth)
  - Raff+ → growth = weaker sugar source → confirms Gal toxicity (GAL7 = no growth)
- Candidate selection was based on its general growth rate in these specific four media conditions favorable towards expected Ssl2 mutations, which should include the extreme downshift of transcription start sites.
  - All candidates followed similar growth patterns to the favorable ones listed above
  - Chosen candidates: 3B, 3E, 2D, 2E, 1E, 2C, 3D & 4E





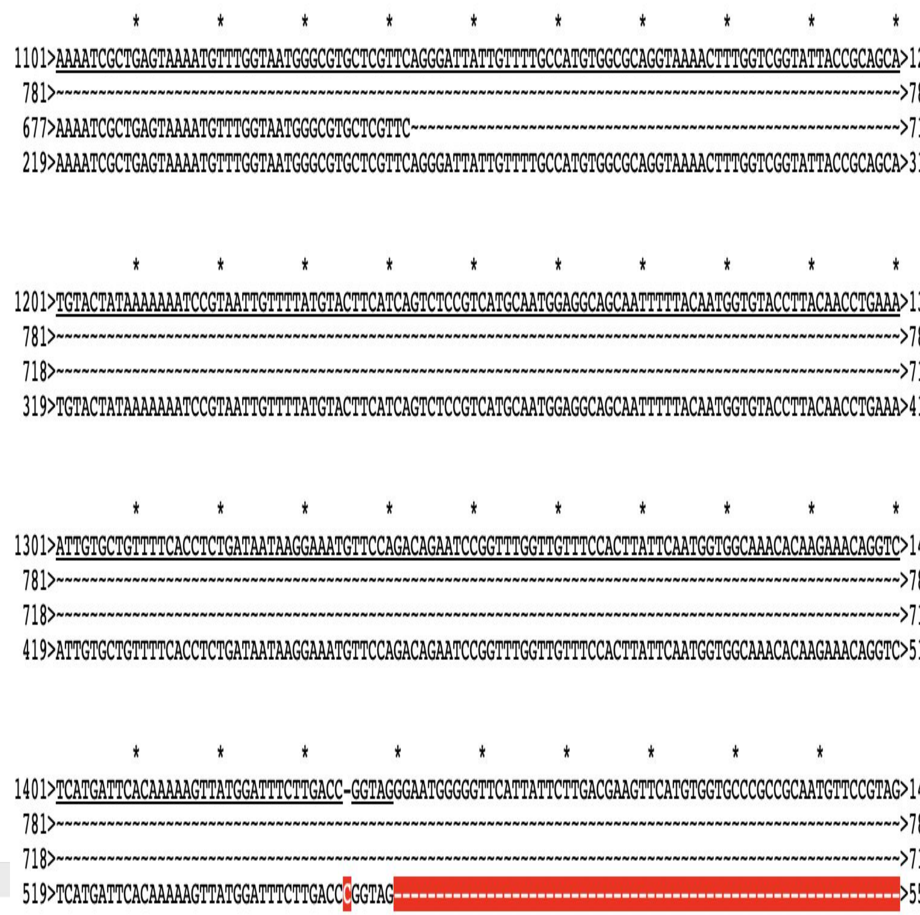
Candidate transcription mutants grown on different media for 2-6 days at 30ºC

Results of Sequencing

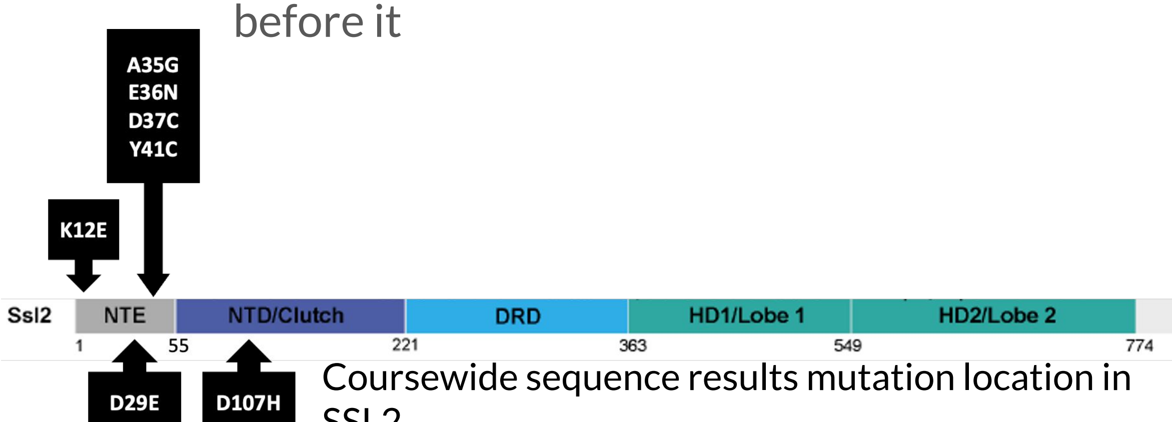
- Despite passing initial mutant screening, no extreme downstream mutations were found in our target sequence of SSL2
  - DRD region has no Ssl2 mutations
- Coursewide sequencing results found that mutations were found to be concentrated in the NTE region
  - Full genome sequencing found that mutated genes were mostly involved in chromatin remodeling
- One insertion mutation was found at the 1434 base pair position, but it was not viable
  - Only one sequence contained the mutation, was only covered by one sequence
  - The base pair is the same as the one before it

Candidate	Gene	Missense mutation	Gene function
UG_aj (cand 2)	*HTB1*	A78P	histone H2B - core histone protein required for chromatin assembly and chromosome function
UG-j (cand 15)	*SPN1*	L249P	Protein involved in RNA polymerase II transcription; also required for histone modifications and splicing
UG_o (cand 12)	*RSC30*	G571D	Component of the RSC chromatin remodeling complex
	SPN1	V199G	Protein involved in RNA polymerase II transcription; also required for histone modifications and splicing
UG_an (cand 5)	*RSC30*	G571D	Component of the RSC chromatin remodeling complex
	HTB1	A78P	histone H2B - core histone protein required for chromatin assembly and chromosome function
UG_c (cand 2B-5_	*RSC30*	G571D	Component of the RSC chromatin remodeling complex
	SPT16	G949R	Chromatin-remodeling protein

Backup candidate full genome sequencing revealed mutations in genes involved in chromatin remodeling



Candidate 1E sequencing results show one insertion



Coursewide sequence results mutation location in SSL2

Coursewide Data

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UG_c (cand 2B-5_	*RSC30*	G571D	Component of the RSC chromatin remodeling complex
	SPT16	G949R	Chromatin remodeling protein

Conclusions & Future Directions

- Our lab group did not find any mutations
  - **Coursewide:** 5 mutations found/identified.
- Effective approach for an experiment
  - Located extreme TSS downshifters
  - Provided additional knowledge of the yeast genome, so over all the experiment was a success
  - Not effective approach for located Ssl2 mutants, rather locating other global defects in the overall genome.
- Mutation does not occur where it was hypothesized to.
  - **Chromatin remodeling** played a much larger role than the experimental team originally anticipated.
- Further experimentation that includes the whole SSL2 gene is needed to get more specific about this region by:
  - Applying how chromatin remodeling affects the mutations of the CKY5369 yeast strain
  - Finding and screening different phenotypes relating chromatin remodeling regions within the genome