Very short fragments containing just the core promoter are sufficient to drive testis-specific gene expression in *Drosophila*.

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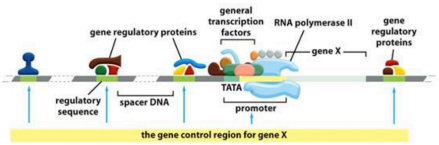
Scientific Rationale

Many genes are exclusively expressed in testes, and this is essential for male fertility.

In *Drosophila*, expression of most of these genes depends on a testis-specific transcription factor complex, tMAC, containing several DNA-binding proteins. The molecular mechanism for how tMAC recognises target promoters is still unknown.

In the textbook view of transcription the core promoter recruits general transcription factors and RNA polymerase, and provides capacity for expression, but specificity comes from distal regulatory elements.



However, in *Drosophila*, there are several examples of testis-specific expression being controlled by just the core promoter, eg a fragment of 100bp flanking the TSS can cause expression of a reporter.

Pedagogic Rationale

This "Advanced Research Methods" module replaces the research project for students in year 3 of a 4 year Integrated Master's degree.

The group project prepares students for a longer, independent, research project in their final year; teaching fundamental lab skills - planning, problem solving, data analysis etc, and core molecular biology techniques.

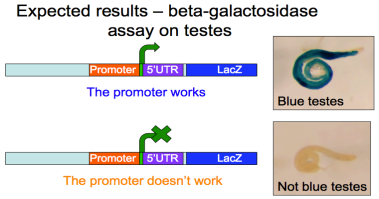
Scientific Approach

To test the hypothesis that testis-specific promoters are typically very short we generated promoter reporter constructs containing sequence flanking the TSS of tMAC-dependent, and tMAC-independent genes.

A second hypothesis based on previous reporter constructs was that translational delay sequences would be found in the 5'UTR of many testis transcripts.

PCR fragments (100, 200, 400bp ish) containing promoter, TSS and 5'UTR from each chosen gene were cloned into the MCS of pCaSpeR-AUG-βGal-attB. This vector lacks a minimal promoter but includes a start codon.

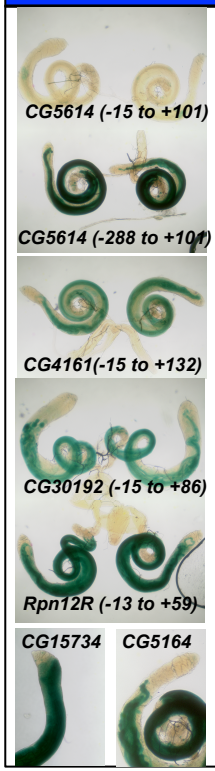
Outsource transgenesis via PhiC31 mediated integration.



Pedagogic Approach

During bioinformatics sessions each student selected a single gene from lists provided. They used database searching to find gene sequence, expression and function information. They designed PCR primers for amplification and cloning. The cloning work was conduced with supervision over 8 days in the lab (2 days per week, 4 weeks). The final 2 days of lab work included testis dissection, staining and imaging. Each student worked independently, but final results were shared.

Scientific Results



Gene	Prom	5'UTR	Result
tomboy20	6	71	not blue
Rpn12R	13	59	blue
CG30192	15	86	blue
CG4161	15	132	blue
CG5614	15	101	not blue
CG12782	19	117	not blue
CG42650	24	68	blue
CG15734	25	71	not blue
CG6441	28	200	not blue
CG6441	28	91	not blue
CG17197	29	88	blue
CG30487	31	62	not blue
CG4956	34	68	blue
CG12307	36	71	not blue
CG17196	41	29	blue
CG12857	49	44	blue
CG14891	49	16	blue
CG32148	51	74	blue
tomboy20	52	71	blue
CG31391	55	68	blue
CG17198	60	64	blue
CG17195	71	15	blue
CG6441	92	200	blue
CG6441	92	91	blue
CG32440	115	121	blue
CG5614	115	101	blue
CG12782	136	117	not blue
CG4956	140	68	blue
CG5755	141	96	blue
CG4438	144	285	blue
CG4161	147	132	blue
CG12307	168	71	blue
CG12857	170	44	blue
CG14891	171	16	blue
CG15734	174	71	blue
CG30487	175	62	blue
CG31391	250	68	blue
CG5614	288	101	blue
CG12782	306	117	blue
CG15734	311	71	blue
CG30192	312	86	blue
CG42650	314	68	blue
CG11043	321	74	not blue
CG12307	334	71	blue
CG30487	343	62	blue
CG32148	356	74	blue

Scientific Conclusions

46 reporter construct transgenics, containing fragments (64bp to 430bp) from 24 different genes.

The shortest construct to drive expression was 64bp (-49 to +16 of *CG14891*). The shortest functional promoter sequence was 13bp (*Rpn12R*).

Almost all (30/32) constructs containing 40bp or more promoter sequence drove expression. 6/13 constructs with 36bp or less of promoter drove expression.

Fragments containing short, or very short promoter sequences can drive both tMAC-dependent (18/19 tested) and tMAC-independent (4/5 tested) genes.

Many of the transgenic testes also showed beta-galactosidase activity only in post-meiotic stages (eg *CG5614)*, consistent with the 5'UTR imposing translational delay on the reporter transcript, others showed no repression (eg *CG15734*).

Pedagogic Conclusions

Admission to the Integrated Master's scheme depends on previous academic performance. The students were generally well motivated, attendance and engagement was high. Many found the lab work challenging initially, but they coped extremely well with the pressures.

The students had varied molecular biology knowledge and experience, due to varied option selections in year 2 of their degree. They developed significant transferable lab skills, which they have used this year in their master's year research project. They also developed group work skills, and made new friendships within the cohort.

This was a fun and rewarding teaching experience for the academic project leaders (we even did some cloning too).

The first 20 authors were the students in the project group. They are listed in alphabetical order by first name, as our lasertag session to determine author order had to be cancelled, and rescheduling became impossible for now due to Covid-19.