Cloning cDNA from oocytes & zygotes

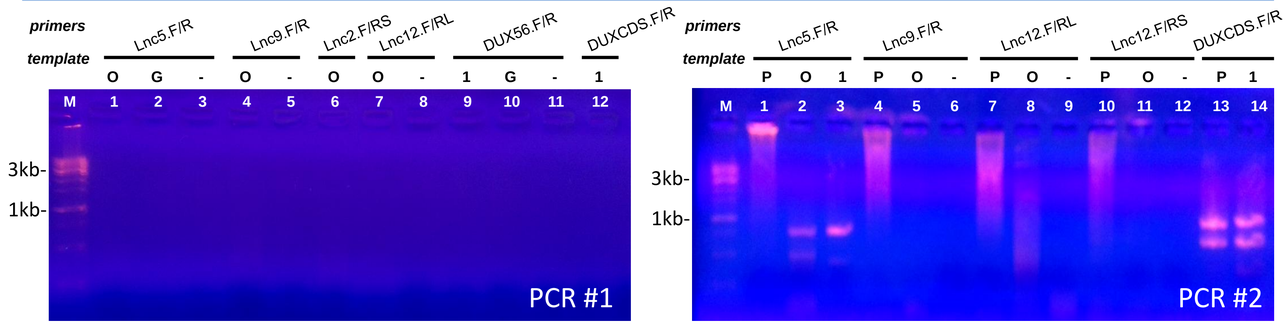
Elizaveta Gubanova, Mariona Latorre, Darja Lepekhina, Marc Masramon, Ilya Popadin

Júlia Canet-Pons, Olga Glazynova, Natalya Markelova, Sergey Shumeyko, Petr Svoboda

ABSTRACT

The theme of our laboratory were genes functioning in the early embryonic development. The control of gene expression during mammal oocyte-to-embryo transition (OET) is a fascinating but poorly understood process. Last year, next generation sequencing revealed many candidate genes which might participate in OET. Among them, four genes, which are active in oocytes and zygotes were selected for functional analysis prior to the school. Three of them are oocyte-specific LncRNA (*Lnc5*, *Lnc9* and *Lnc12*) selected during a bioinformatics survey of the maternal transcriptome. The last is Dux, a protein-coding gene which might be involved in the early phase of the zygotic genome activation. In order to study function of the selected genes, it is necessary to clone their cDNA, which will be used in further experiments. Accordingly, the project involved the production of recombinant DNA in bacteria using genetic engineering techniques. First, we used the polymerase chain reaction (PCR) to amplify selected genes. Obtained PCR products were inserted into pcDNA3.1/CT-GFP-TOPO plasmid using GFP Fusion TOPO TA Expression Kit. Produced plasmid DNA was quantified and tested for the presence (and orientation) of inserts by a digestion with restriction enzymes. Overall, we successfully cloned *Lnc5* cDNA and several cDNA fragments which need further characterization. Since the pcDNA3.1/CT-GFP-TOPO plasmid contains the coding sequence of green fluorescent protein (GFP), we could transform it into a living cell to observe the expression of protein-coding genes (*Dux*). But unfortunately, the time and conditions did not allow us to do so. In the future, the results of our study will be used to study the functions of selected genes in the laboratory in Prague.

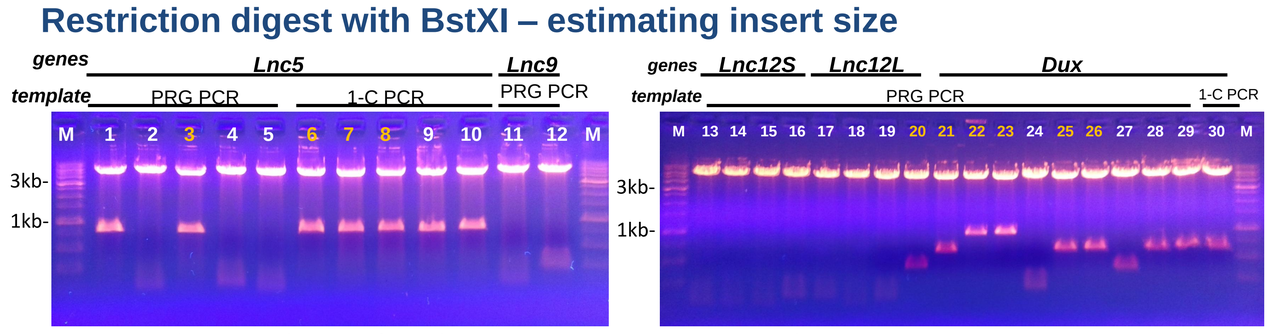
AMPLIFICATION OF *Lnc5*, *Lnc9*, *Lnc12* & *Dux* INSERTS



Template abbreviations: O – ovary cDNA, 1 – 1-cell cDNA , G – mouse genomic DNA, P – diluted PCR product from Prague (100x), PCR program: 36 cycles, denaturation 94°C 30 sec, annealing 56°C 30 sec, extension 72°C 2 min. Resolved in 1% agarose gel, 100V, ~ 45 min. PCR#1 did not work because the final dNTP concentration was accidentally 10x higher (2µM) than recommended (200 nM).

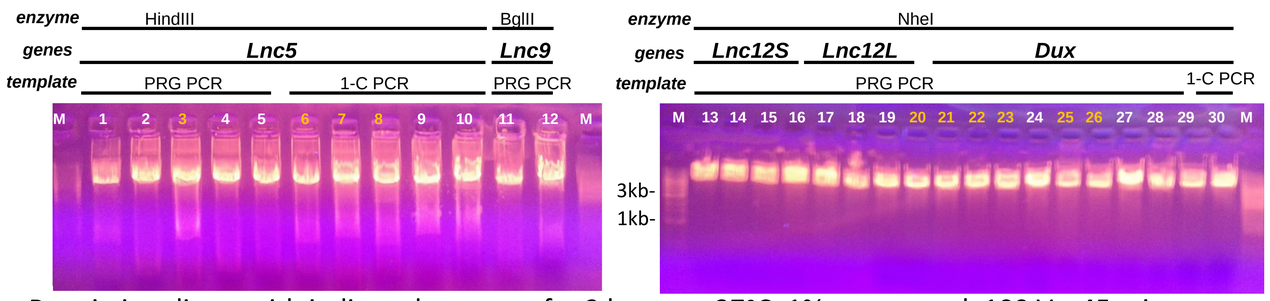
ANALYSIS OF INSERTS IN pcDNA3.1/CT-GFP-TOPO

Restriction digest with BstXI – estimating insert size



Restriction digest with BstxI for 2 hours at 37°C, 1% agarose gel, 100 V, ~45 min.

Restriction digest – examining insert orientation



Restriction digest with indicated enzymes for 2 hours at 37°C, 1% agarose gel, 100 V, ~45 min.

PROJECT WORKFLOW

Assembling transcript models of genes

↓

Estimating PCR product size
Amplification of cDNA (PCR)

↓

Analysis of PCR products (electrophoresis)

↓

Selection of products for cloning
Insertion of PCR products into vectors
Transformation of bacteria

↓

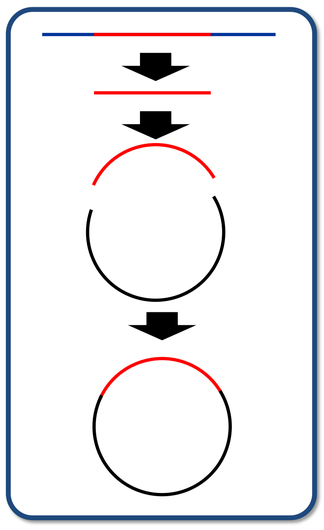
Selection of colonies & liquid culture

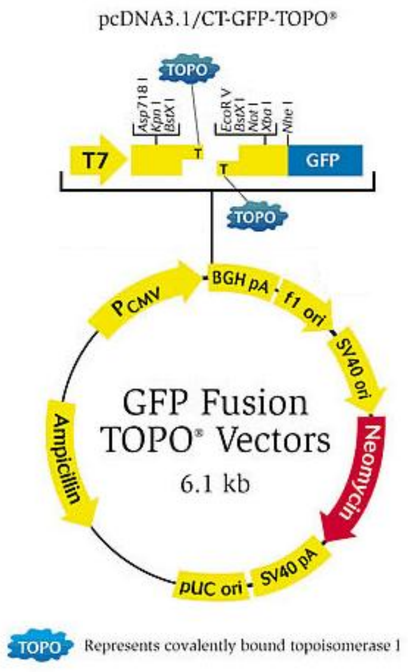
↓

DNA isolation
DNA quantification
Restriction digest analysis

↓

Sending DNA to Prague



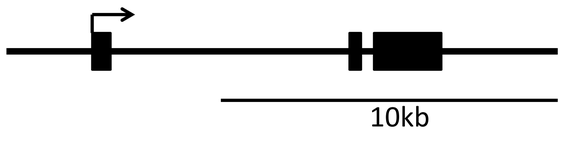


OVERVIEW OF ALL OBTAINED CLONES

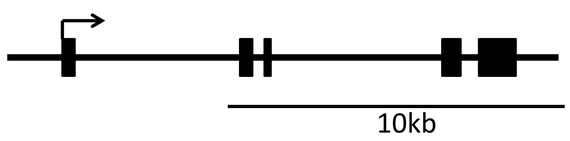
clone	conc. (ng/uL)	A260/280	PCR primers	Insert (bp)		Conclusion
				Expected	Observed	
1	316,7	1,91	Lnc5.F/R	737	~750	Lnc5 antisense
2	266',1	1,93	Lnc5.F/R	737	<250	Unknown
3	**272,6**	**1,9**	**Lnc5.F/R**	**737**	**~750**	**Lnc5 sense**
4	235,2	1,9	Lnc5.F/R	737	<250	Unknown
5	292,4	1,82	Lnc5.F/R	737	<250	Unknown
6	**333,6**	**1,88**	**Lnc5.F/R**	**737**	**~750**	**Lnc5 antisense**
7	**177,1**	**1,89**	**Lnc5.F/R**	**737**	**~750**	**Lnc5 sense**
8	**184,0**	**1,89**	**Lnc5.F/R**	**737**	**~750**	**Lnc5 antisense**
9	215,9	1,90	Lnc5.F/R	737	~750	Lnc5 sense
10	266,6	1,90	Lnc5.F/R	737	~750	Lnc5 sense
11	202,9	1,87	Lnc9.F/R	1845	<250	Unknown
12	229,7	1,86	Lnc9.F/R	1845	<250	Unknown
13	277,6	1,89	Lnc12.F/RS	583	<250	Unknown
14	388,5	1,74	Lnc12.F/RS	583	<250	Unknown
15	241,2	1,77	Lnc12.F/RS	583	<250	Unknown
16	354,3	1,88	Lnc12.F/RL	1686	<250	Unknown
17	315,6	1,81	Lnc12.F/RL	1686	<250	Unknown
18	206,2	1,81	Lnc12.F/RL	1686	<250	Unknown
19	321,8	1,89	Lnc12.F/RL	1686	<250	Unknown
20	**311,3**	**1,83**	**Lnc12.F/RL**	**1686**	**~500**	**Unknown**
21	**262,7**	**1,91**	**DuxCDS.F/R**	**2041/2091**	**~700**	**Unknown**
22	**304,9**	**1,90**	**DuxCDS.F/R**	**2041/2091**	**~1000**	**Unknown**
23	**388,1**	**1,81**	**DuxCDS.F/R**	**2041/2091**	**~1000**	**Unknown**
24	383,6	1,86	DuxCDS.F/R	2041/2091	<250	Unknown
25	**370,5**	**1,84**	**DuxCDS.F/R**	**2041/2091**	**~600**	**Unknown**
26	**212,0**	**1,90**	**DuxCDS.F/R**	**2041/2091**	**~600**	**Unknown**
27	334,6	1,87	DuxCDS.F/R	2041/2091	~400	Unknown
28	223,6	1,85	DuxCDS.F/R	2041/2091	~600	Unknown
29	196,2	1,93	DuxCDS.F/R	2041/2091	~600	Unknown
30	192,5	1,92	DuxCDS.F/R	2041/2091	~600	Unknown

GENES OF INTEREST

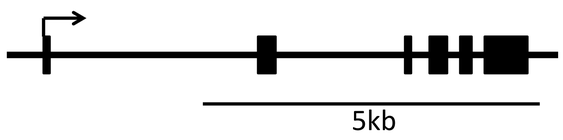
Lnc5 chr5:150,687,800-150,724,284



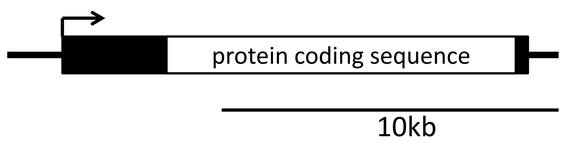
Lnc12 chrX:7,617,568-7,658,006



Lnc9 chr19:57,567,753-57,591,144



Dux chrX:7,617,568-7,658,006



SUMMARY

- *Lnc5* cDNA was successfully cloned in both directions.
- *Lnc9*, *Lnc12*, *Dux* cDNAs were not cloned because of suboptimal conditions for cDNA amplification by PCR.
- One has to focus while pipetting (all the time, actually).
- It is important to control all possible experimental conditions.
- Nucleotides & voltage really matter!