Pràcticum I. Final Report

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1. Introduction

1.1 Mission of the CRG

The Centre for Genomic Regulation (CRG) is an international biomedical research institute of excellence, created in December 2000. It is a non-profit foundation funded by the Catalan Government through the Departments of Economy & Knowledge and Health, the Spanish Ministry of Economy, Industry and Competitiveness, the "la Caixa" Banking Foundation, and includes the participation of Pompeu Fabra University.

The mission of the CRG is to discover and advance knowledge for the benefit of society, public health and economic prosperity. The CRG believes that the medicine of the future depends on the ground-breaking science of today. This requires an interdisciplinary scientific team focused on understanding the complexity of life from the genome to the cell to a whole organism and its interaction with the environment, offering an integrated view of genetic diseases.

1.2 Genome Architecture Lab

The Genome Architecture Laboratory was established in 2012 by Dr Guillaume Filion, who also supervised the work presented in this report.

During this period, there has been established several research lines, but they have in common one thing: they are all focused on the understanding the chromatin and topology effects on gene expression and nuclear biology. As the sequence is not enough to explain the variation in gene expression, there is a need to identify the other factors involved in transcription.

The most important research lines, among others not named, are:

- Position-dependent effects on housekeeping promoters in *Drosophila*.
- Position effects on HIV integration and expression.
- Polymer physics of chromatin and properties of transcription factors mechanics.
- Nuclear organization by progesterone receptor and oestrogen receptor in breast cancer cells

1.3 Purpose of the project

The aim of the project (not only of this practicum) is to understand the factors involved in the expression of a housekeeping promoter integrated into heterochromatin regions (Corrales *et al.* , 2017; Corrales, 2017).

To do that, I made use of TRIP technology. Briefly, TRIP consists in the use of a library of barcoded reporters (reporters with a 20-mer random sequence) that are integrated randomly in the genome using a transposase. This system allows us to relate the expression of each unique reporter to a location in the genome. Using this technology enable us to study the position effects in a high-throughput way (Akhtar *et al.* , 2013, 2014).

To address the question below, I cloned a barcoded before the poli-A tail. I amplified the library using electrocompetent *E. coli* cells and cotransfected it into Kc167 cells (a Drosophila cell line) with the *Sleeping Beauty* transposase (Mátés *et al.* , 2009). After growing the cells, I FACS selected them and seeded 1 cell/well in a 96-well plate and grow them. The goal of this process is to get isogenic populations (cells that come from a single cell progenitor) that putatively contain at least 1 integration.

After growing these clones, I harvested the cells and extract the genomic DNA in order to map the barcodes in the genome. The will of that is to get a clone with at least 2 mapped integrations, 1 in euchromatin and the other one in heterochromatin.

Thw workflow is summarized in figure 1

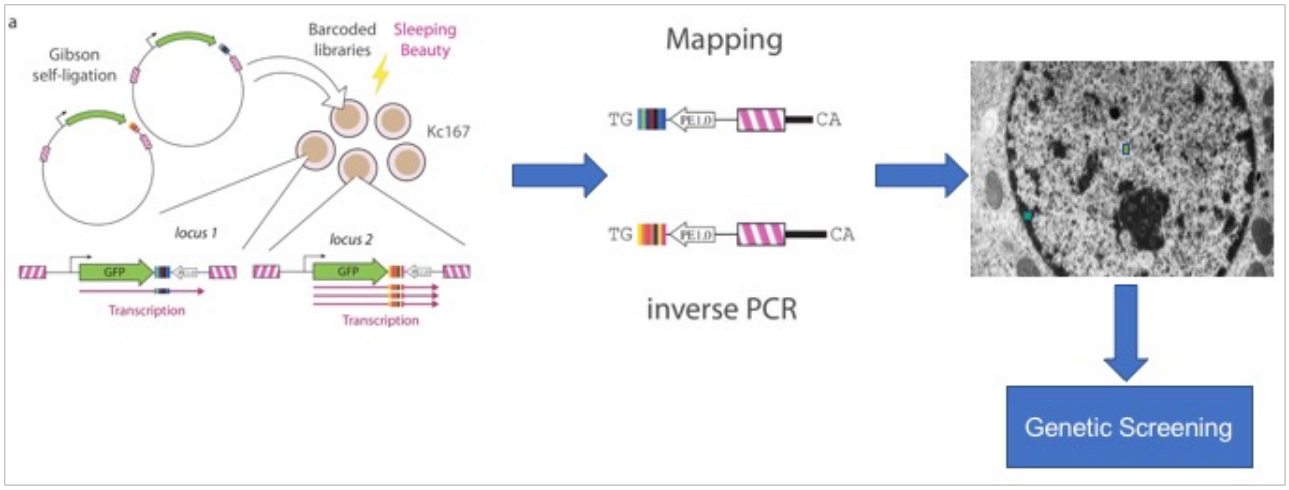

Figure 1: Workflow of the project. The green squares represent the GFP integrations

This will allow us to screen this cells for factors involved in heterochromatic expression of this promoter of interest.

1.4 Position effects variegation

As this practicum aims to disentangle the mechanisms of a position effects event, it is important to properly define it.

Defined by its pioneer Sarah Elgin, position effects variegation (PEV) results when a gene normally in euchromatin is juxtaposed with heterochromatin rearrangement or transposition. When heterochromatin packaging spreads across the heterochromatin/euchromatin border, it causes transcriptional silencing in a stochastic pattern. PEV is intensely studied in *Drosophila* using the *white* gene (Elgin & Reuter, 2013).

Traditionally, the definition of heterochromatin was mainly cytological. Heterochromatin was defined as such regions that appeared electrodense in transmission electron microscopy experiments.

1.5 The TRIP technology

2. Objectives and activities

2.1 Aim of the practicum

2.2 Barcoding PCR

The first step is to do the barcoding PCR according to this protocol:

Reagent
10 ng plasmid
2.5 uL primer GAT155
2.5 uL primer GAT385
10 uL GC buffer 10x
1 uL dNTPs
0.5 uL Phusion Polymerase
32.5 uL water

with this programme:

T	Time
98C	2 min
98C	15 sec
60C	20 sec
72C	5 min
x28 cycles	
72C	2 min

Check the outcome of PCR with 1% agarose gel.

2.3 DpnI digestion

The recipe is:

Reagent
45 uL BCR PCR product
5 uL CutSmart Buffer 10x
1 uL DpnI enzyme

1h at 37C and 20 min at 80C.

2.4 MinElute kit purification

1. Add 5 volumes of PB buffer for 1 volume of PCR product and 10 uL of NaAc 3M.
2. Put the column in a 2 mL collection tube.
3. Spin the column 1 min at 13,000 rpm and discard the flow-through.

4. Add 750 uL of PE buffer and spin at 13,000 rpm for 1 min. Discard the flow-through.
5. Spin for 2 min and replace the collection tube (1 mL tube).
6. Elute in 10+10 uL EB buffer. Wait 5 min and spin for 1 min.

2.5 Gibson Cloning

1. 5 uL of template DNA
2. 5 uL water
3. 10 uL Gibson mix

Incubate this mix 1h at 50ºC.

2.6 EtOH Purification

1. Add 2.5 volumes of EtOH.
2. Add 1/10 of NaAc 3M and 2 uL of glycogen.
3. Immerse in EtOH + carbonic niege to freeze and precipitate DNA.
4. Spin for 30 min at 16,000 rpm at 4ºC.
5. Discard the supernatant.
6. Wash the pellet with 75% EtOH and spin for 10 min at 16,000 rpm at 4C.
7. Ressuspend the pellet in 10 uL of water.

2.7 Transformation of high electrocompetent E. coli cells

1. Thaw the aliquot of E. coli in ice.
2. In 0.1 cm cuvettes, pipette 25 uL of E. coli suspension and 2.5 uL of purified plasmid.
3. Electroporate with the corresponding programme.
4. Add 1 mL of recovery medium (aka SOC media).
5. Pool the transformed cells in a 15 mL falcon.
6. Prepare 3 LB + Amp agar plates and a seriate dilution of the culture (1/1000,1/2000,1/4000):

Dilution	Culture volume	Final volume
1/1000		200 uL
1/2000		200 uL
1/4000		200 uL

Plate this 200 uL in this plates and culture overnight at 37ºC.

1. Transfer the rest of the culture into 500 mL of liquid LB+Amp 1x (500 uL) and culture ON at 37C.

2.8 MaxiPrep NucleoBond Xtra Midi kit

1. Culture E. coli cells at 37C and at 180 rpm overnight.
2. Harvest cells and centrifuge at 4500g at 4C for 15 min.
3. Ressuspend the pellet in 12 mL of RES buffer, add 12 mL LYS buffer and wait 5 min at RT.
4. Equilibrate the column with 25 mL of EQU buffer.
5. Add 12 mL of NEU buffer to the lysate.
6. Mix the tube and load the lysate into the column.
7. 1st wash. Add 15 mL of buffer EQU.
8. Discard the filter.
9. 2nd wash. Add 25 mL of buffer WASH.
10. Add 25 mL of ELU buffer.
11. Add 10.5 mL of isopropanol, vortex and centrifuge at 4000 rpm for 1h at 4ºC.
12. Discard the supernatant and add 4 mL of 70% EtOH and centrifuge at 4000 rpm at 4C for 15 min.
13. Discard the supernatant and ressupend the pellet in 100 uL of nuclease-free water.
14. Quantify the library in a Nanodrop and leave the prep at 1ug/mL.

conc ng/uL	260/280	260/230

3. Cell culture

Cells grow at 25C with Schneider's Drosophila medium supplemented with 5% FBS.

To pass the cells, I take 5 mL of the culture + 15 mL of medium.

4. Transfection of Kc167 cells by electroporation

They are needed 20 milions cells per transfection.

1. Prepare as much plates as you need (1 plate for transfection) in order to get 20 milions cells at the day of transfection.
2. Spin cells at 800g for 3 min.
3. Remove the supernatant and resuspend the cells in 800 uL x number of transfections.
4. Pipette 20 ug of DNA in an electroporation cuvette (Biorad, 0.4 cm gap).

5. Add 800 uL of cell suspension and pipette up & down 2-3 times.
6. Electroporate at 1000 uF and 250 V.
7. Wait 5 min.
8. Transfer the cells into a 10 cm dish containing 10 mL of Schneider medium + 5% FBS previously warmed at RT.
9. Grow cells at 23ºC.

5. Kc167 conditioned medium

1. Spin the culture at 200g for 5 min.
2. Take the supernatant and filter it with a 45 um filter carefully.
3. Dilute the filtrate 1:1 with fresh Schneider Medium.
4. Store at 4ºC.

6. Results and Conclusion

7. Self-assessment

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Addendum

Primer sequences