

Etudier l'activité Enhancer de milliers de séquences avec le STARRseq

Réunion biologie

06.03.26

Amélie Escandell

Méthode

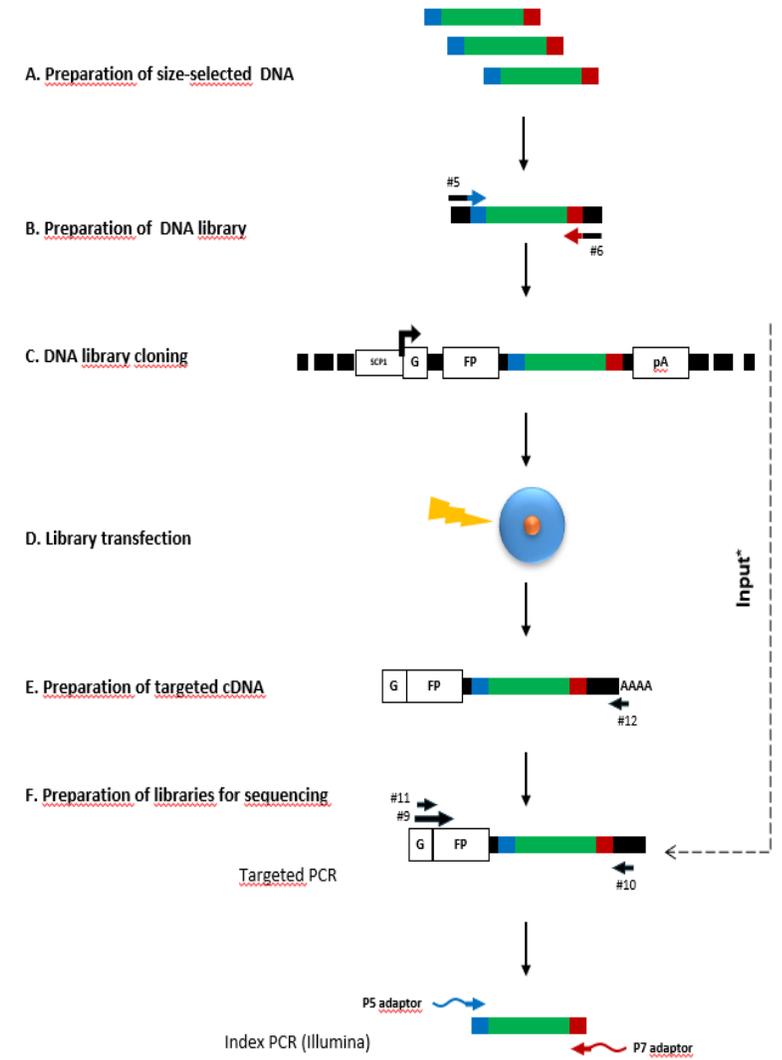
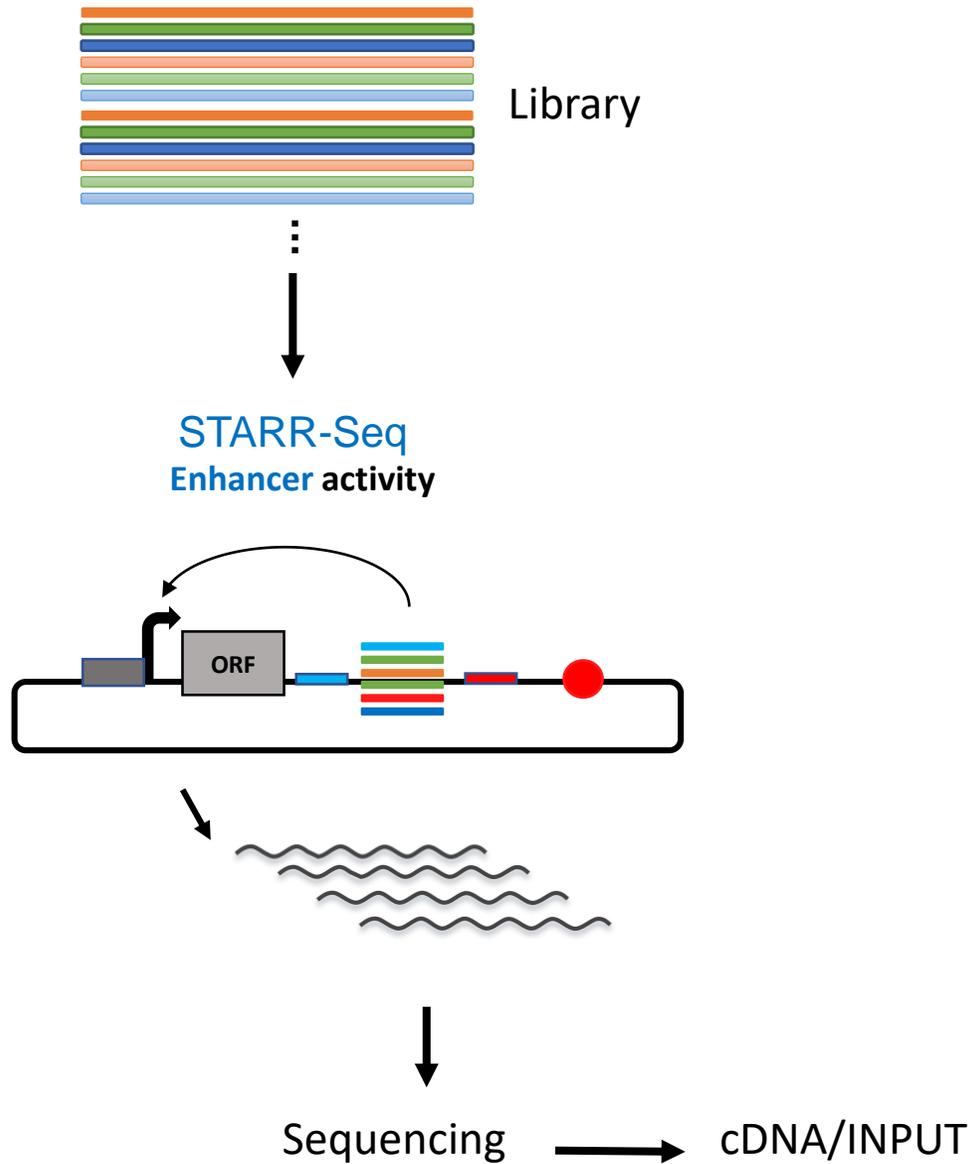
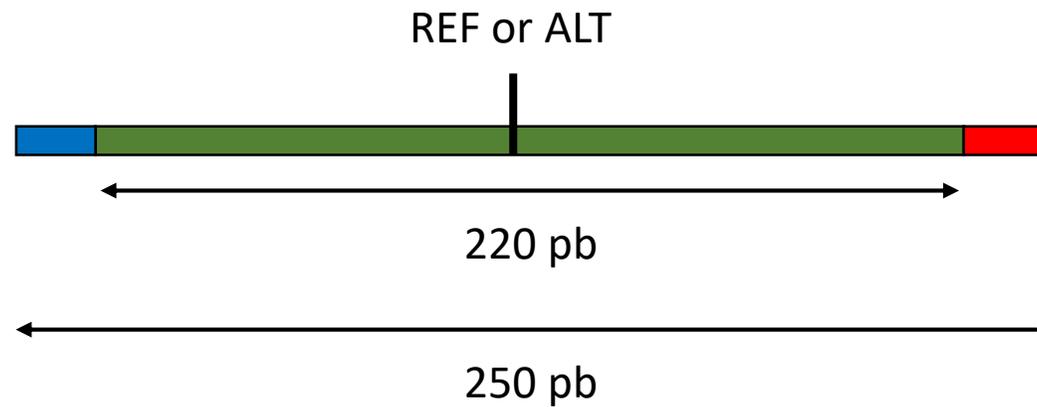
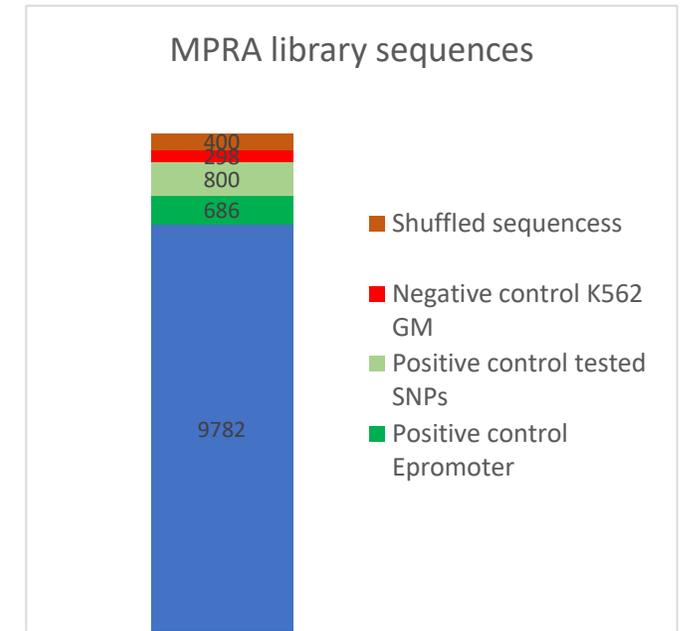


Figure 1: Overview of the enhancer STARR-seq procedure. See main text for details. Primers are listed in table 1. *For input amplification primer #9 is replaced by #11. The blue and red boxes indicate the Illumina adaptors.

Design de la librairie

Positive-control-Epromoter	sequences	104 sequences for Epromoters active in ≥ 5 cell lines
	686	582 sequences for Epromoters active in K562 or GM12878
Positive-control-tested-SNPs	sequences	Top 100 SNPs from Abell2022(GM12878) and Arensbergen2019(K562) ranking by allelic effect pvalue
	800	200 SNPs with Ref/Alt alleles, forward/reverse strand = 800 sequences
legControl-K562-GM12878-lowAct	sequences	Low regulatory activity($0.9 < FC < 1.1$) in K562/GM12878 CapSTARR-seq
	298	exclude Epromoters in other cell lines
Shuffled_sequence	sequences	scramble(shuffling) sequences from positive control (MPRA tested)
	400	shuffled 200 sequences contained SNPs, pseudo forward/reverse = 400 sequences
total	11966	



Production de la librairie chez TWIST

Oligo Pools Pricing

Cloned oligo pools
 Oligo pools
 🎓 Academic Pricing Applied

For pools with more than 696,000 sequences please contact your account manager or customersupport@twistbioscience.com for pricing

Tier (Pool size)	20 - 120nt	121 - 150nt	151 - 200nt	201 - 250nt	251 - 300nt
Tier 1 (2-100 Oligos)	\$320.00 \$400.00	\$372.80 \$466.00	\$416.00 \$520.00	\$551.20 \$689.00	\$824.00 \$1,030.00
Tier 2 (101-500 Oligos)	\$640.00 \$800.00	\$746.40 \$933.00	\$832.00 \$1,040.00	\$1,103.20 \$1,379.00	\$1,648.00 \$2,060.00
Tier 3 (501-1,000 Oligos)	\$960.00 \$1,200.00	\$1,120.00 \$1,400.00	\$1,248.00 \$1,560.00	\$1,654.40 \$2,068.00	\$2,472.00 \$3,090.00
Tier 4 (1,001-2,000 Oligos)	\$1,280.00 \$1,600.00	\$1,493.60 \$1,867.00	\$1,664.00 \$2,080.00	\$2,205.60 \$2,757.00	\$3,296.80 \$4,121.00
Tier 5 (2,001-6,000 Oligos)	\$1,920.00 \$2,400.00	\$2,240.00 \$2,800.00	\$2,496.00 \$3,120.00	\$3,308.80 \$4,136.00	\$4,944.80 \$6,181.00
Tier 6 (6,001-12,000 Oligos)	\$2,496.00 \$3,120.00	\$2,995.20 \$3,744.00	\$3,244.80 \$4,056.00	\$4,118.40 \$5,148.00	\$6,155.20 \$7,694.00
Tier 7 (12,001-18,000 Oligos)	\$3,244.80 \$4,056.00	\$3,893.60 \$4,867.00	\$4,218.40 \$5,273.00	\$5,355.20 \$6,694.00	\$8,003.20 \$10,004.00
Tier 8 (18,001-24,000 Oligos)	\$4,218.40 \$5,273.00	\$5,061.60 \$6,327.00	\$5,484.00 \$6,855.00	\$6,961.60 \$8,702.00	\$10,404.80 \$13,006.00
Tier 9 (24,001-30,000 Oligos)	\$5,484.00 \$6,855.00	\$6,580.00 \$8,225.00	\$7,129.60 \$8,912.00	\$9,052.00 \$11,315.00	\$13,528.00 \$16,910.00
Tier 10 (30,001-36,000 Oligos)	\$5,629.60 \$7,037.00	\$6,755.20 \$8,444.00	\$7,319.20 \$9,149.00	\$9,292.00 \$11,615.00	\$13,887.20 \$17,359.00
Tier 11 (36,001-42,000 Oligos)	\$6,192.80 \$7,741.00	\$7,431.20 \$9,289.00	\$8,051.20 \$10,064.00	\$10,220.00 \$12,775.00	\$15,274.40 \$19,093.00
Tier 12 (42,001-48,000 Oligos)	\$6,812.00 \$8,515.00	\$8,174.40 \$10,218.00	\$8,856.00 \$11,070.00	\$11,240.80 \$14,051.00	\$16,800.00 \$21,000.00
Tier 13 (48,001-54,000 Oligos)	\$7,493.60 \$9,367.00	\$8,992.00 \$11,240.00	\$9,741.60 \$12,177.00	\$12,364.80 \$15,456.00	\$18,480.00 \$23,100.00
Tier 14 (54,001-60,000 Oligos)	\$8,243.20 \$10,304.00	\$9,891.20 \$12,364.00	\$10,716.00 \$13,395.00	\$13,602.40 \$17,003.00	\$20,328.80 \$25,411.00
Tier 15 (60,001-72,000 Oligos)	\$8,655.20 \$10,819.00	\$10,385.60 \$12,982.00	\$11,252.00 \$14,065.00	\$14,283.20 \$17,854.00	\$21,347.20 \$26,684.00
Tier 16					

Close

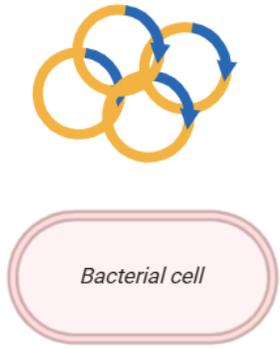
Amplification de la librairie

Table 1: Primer sequences

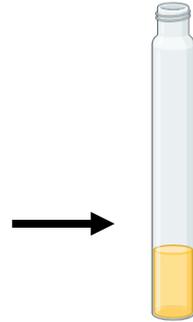
Custom Primers	Name	Sequence (5' – 3')	Purification
5	Capstarr_seq_F	TAGAGCATGCACCGGACACTCTTCCCTACACGA CGCTCTTCCGAT*C*T	HPLC
6	Capstarr_seq_R	GGCCGAATTCGTGAGTGACTGGAGTTCAGACGT GTGCTCTTCCGAT*C*T	HPLC



Culture bactérienne

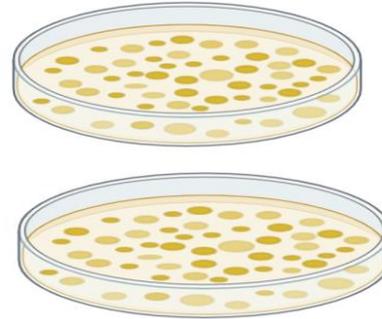


Bactéries MegaX DH10B
(Electrocompétentes)

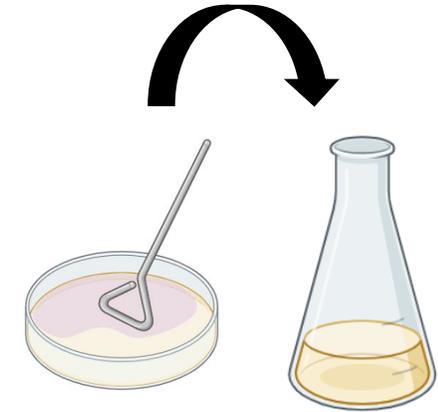


1h milieu
SOC

500 μ L X2 grandes boites



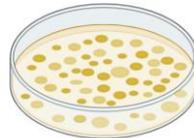
400 mL culture
Milieu TB + carb



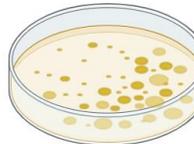
Pour contrôler la complexité

Compter les colonies

Calculer la quantité de culture
nécessaire pour 100K-500K colonies



1 μ L



0,1 μ L



0,01 μ L

Transfection des cellules

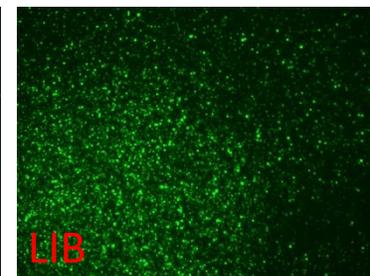
Lignée cellulaire	Système de transfection	N cellules par Réplicat	Quantité ADN par million de cellule	Extraction ARN
K562 (erythroleucémie)	Neon	5M	5µg	Après 24h
A549 (adénocarcinome)	Lipofectamine	5M	5µg	Après 24h
CCRF-CEM (Leucémie T)	Neon	10M	5µg	Après 24h
GM12878 (Lymphoblastoïde B)	Neon	10-20M	3µg	Après 6h
MEC-1 (Lymphome B)	Neon	10M	5µg	Après 24h
HG-3 (Lymphome B)	Neon	10M	5µg	Après 24h
Karpas-422 (Lymphome B)	Neon	10M	5µg	Après 24h (max 10% viabilité)

4 réplicats

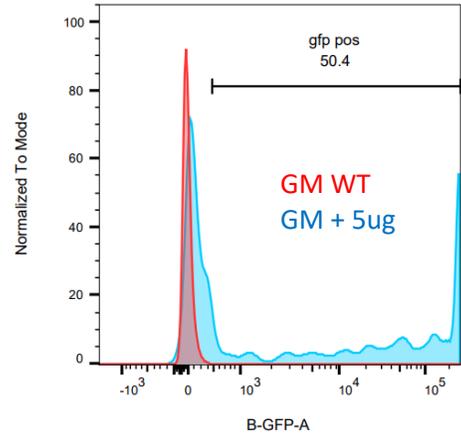
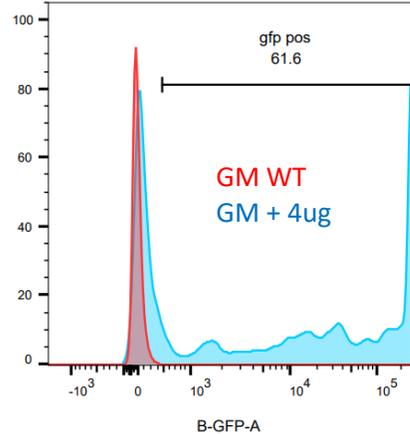
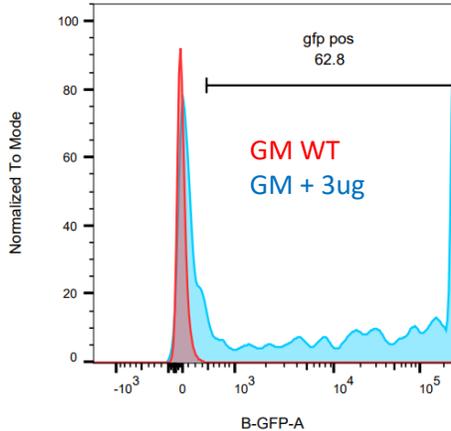
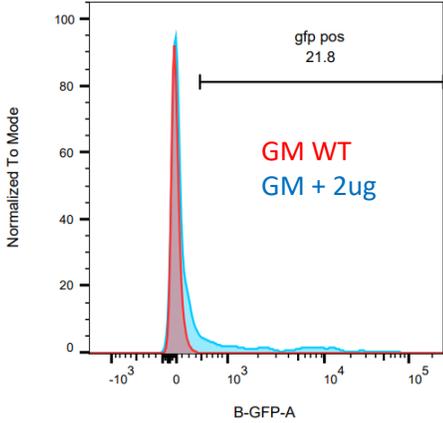
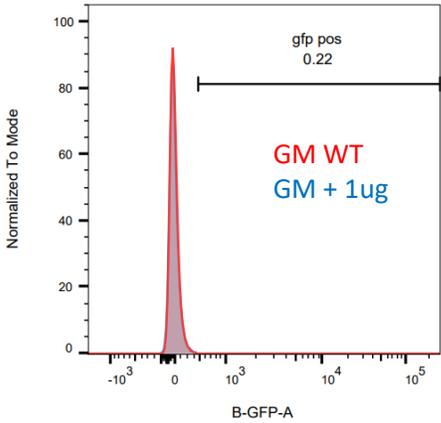
Contrôles :

1 contrôle WT

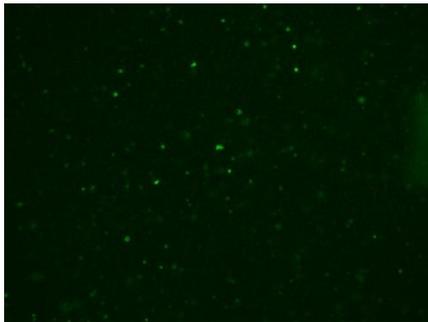
1 contrôle Plasmide vide



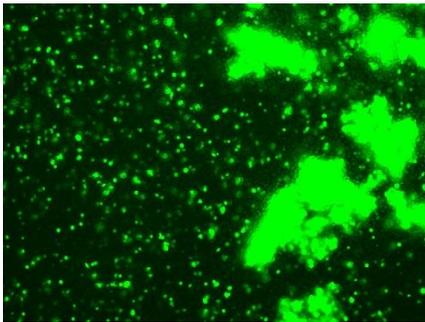
Ex : GM12878 Neon 10ul



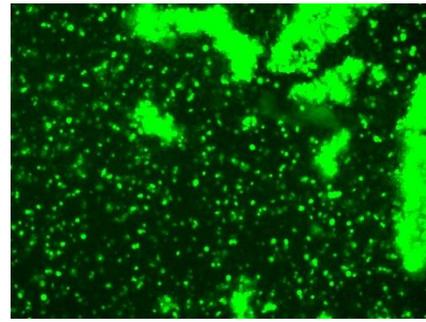
24h Vivantes : 60%



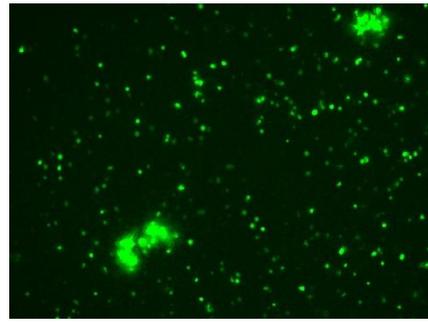
Vivantes : 40%



Vivantes : 25%



Vivantes : 19%

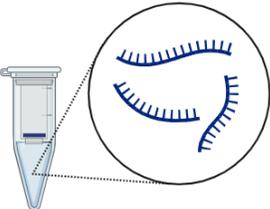


Vivantes : 10%

6h

Vivantes : 40%

Préparation pour le séquençage



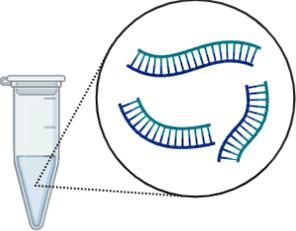
Extraction
ARN tot

Kit : Rneasy mini kit
part 1 & 2 (Qiagen)



Isolation
ARNm

Kit : Dynabeads
mRNA purification



Synthèse
cDNA

Kit : Superscript III
first strand synthesis

Extraction
ADNg/INPUT

12	RT primer	CAAACATCAATGTATCTTATCATG	HPLC
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Préparation pour le séquençage

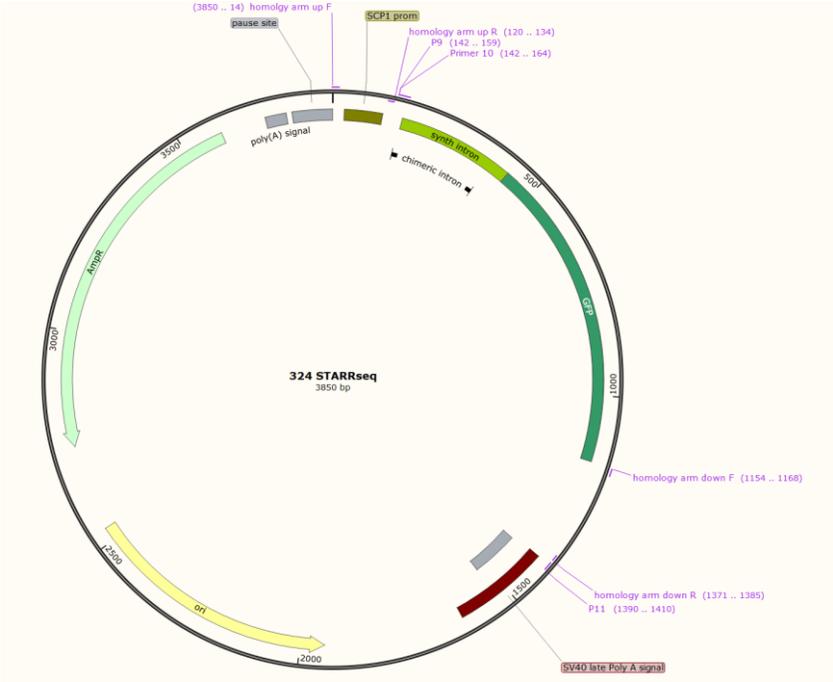
PCR1

cDNA

P9 + P10

INPUT

P11 + P10



PCR2 (indexing PCR)

Un couple de primer i5 et i7 unique par échantillon

Kit : NEBNext Multiplex Oligos for Illumina



Purif sur bille

Kit : Ampure XP beads



Séquençage Illumina

Séquençage en PE
(150pb gauche et droite)

LIB : 20M de reads (10M PE)
INPUT et cDNA : 10M de reads (5M PE)

9	<u>Targeted_lib_F</u>	GGGCCAGCTGTTGGGGTG*T*C*C*A*C	HPLC
11	<u>Targeted_input_F</u>	GGGCCAGCTGTTGGGGTG*A*G*T*A*C	HPLC
10	<u>Targeted_R</u>	CTTATCATGTCTGCTCGA*A*G*C	HPLC

Documentation en ligne

Guide détaillé de toutes les étapes critiques pour la préparation des librairies et l'analyse des données MPRA

Bookdown guide

https://gokhmanlaborganization.github.io/MPRA_QC_book/

Liste de script pour le Quality Control et l'analyse bio informatique :

https://github.com/GokhmanLabOrganization/MPRA_QC_analysis