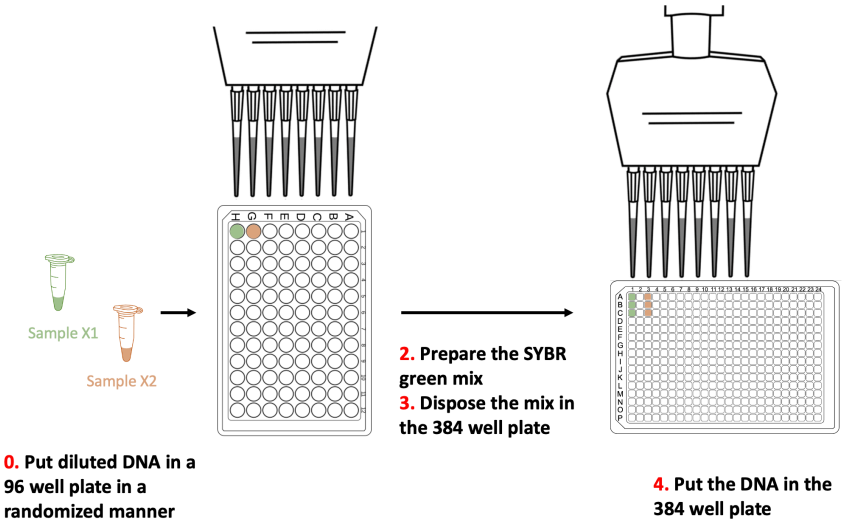
Melting curve analysis *rec-1* protocol

A. Prepare a 384 well qPCR plate

Per well we need:

- Total volume =15 µl; SYBR= 7.5 µL
- primers concentration = 0.3 µM;
- DNA quantity = 15 ng

0. If many samples, you usually want to transfer your DNA samples first to a 96 well plate (to use the multichannel pipet to fill the 384 plate afterward).
    - *The volume should be equal to the volume of DNA sample per well times the number of technical replicates with a generous surplus.*
    - *You should do **randomize** the sample on the plate (at least avoid generation structure on the plate).*
    - *This can be done days before the qPCR and stored at -20°C.*
    - *warning: the distance between two tips of the multichannel pipet correspond to two wells of a 384 well plate.*



1. Prepare templates

96 well:

	H	G	F	…
1	X1	X2	X3	X4
2				
3				
4				
…				

384 well:

	1	2	3	4	…
A	H1		G1		E1
B	H1		G1		E1
C	H1		G1		E1
…					

Here you may just want to indicate the corresponding well of the 96 plate.

1. Prepare your plate design (example and virgin template at the end).

2. Prepare your mix:

	Volume (µL) for one well	x n = ….
SYBR green Master mix	7.5	
Primer rec1indel F 10 µM	0.45	
Primer rec1indel R 10 µM	0.45	
H20 nulcease free	0.6	

*n = number of samples * number of technical (qPCR) replicates + surplus*

3. Put 9 µL of mix in each well

4. Add 6 µL of genomic DNA diluted at 2.5 ng /µJ.

Note: There are other alternatives to reach the desired primers concentration of 0.3 µM and DNA quantity of 15 ng with a total volume of 15 µL. **Example**:

1. Prepare your plate design (example and virgin template at the end).

2. Prepare your mix (with a surplus)

	Volume (µL) for one well	x n = ….
SYBR green Master mix	7.5	
Primer rec1indel F **100** µM	**0.045**	
Primer rec1indel R **100** µM	**0.045**	

3. Put **7.5 µL** of mix in each well

4. Add **7.5 µL** of genomic DNA diluted at **2 ng / µL**. (!) Do not forgot the calibration samples to recover frequency if needed.

Advice:

- You should randomize samples on the 96 well plate.
- if possible, disperse the technical replicate on the 384 qPCR plate
- (!) Do not forgot the calibration samples to recover frequency if needed.
- If the experiment requires more than one qPCR plate, at least one technical replicate of the sample should be present on each qPCR plate. If you do several plates, do different 384 well template to vary positions.

B. Run 384 well qPCR plate on the LightCycler480 instrument

1. Centrifugate the qPCR plate at 1000 – 1800 rpm during 1min

2. Put the plate in on the LightCycler480 instrument (2th floor, local 205, "Salle d'instrumentation") and run the qPCR program:

   I. Turn on the computer (*login: operator; password: LC480*)
   II. Launch the Exor database and the **Roche480 software** (*login: Roche480; password: Roche480*).
   III. Click "New experiment from template" and choose "melting_curve_analysis_rec1_teotonio" [35 cycles: 95°C ⇔ 60°C; then melting: 60°C to 95°C at 0.02°C/s and 25 acquisitions/°C].
   IV. Run and name the file (for consistency please name as: "YYMMDD_INITIAL_description". Ex: "*230210_TP _competion_salt*").

C. Extract the data

1. Click “Open existing object” → Roche480, then select the file.
2. Click on  and export the .ixo file so you can read it on the Roche480 installed on the lab computers [login: admin ; password: LightCycler480]
3. On the lab computer, click on  and import the .ixo file.
4. Optional (but you should): Go in Data and right click on the amplification curve to save amplification data as a .txt file (export chart → data → Format: text).
5. Go in Analysis → “Melt Curve Genotyping”. Select all well and right click on the raw melting curve plot to save melting data as a .txt.file (export chart → data → Format: text).

D. Analyse the data on R

1. To analyze the data, you need to prepare a design indicating the samples details and corresponding well (see melting_curves_analysis/design_example in github).
2. Use the HRM function (in function_melting_analysis.R) to extract metrics used to infer the *rec-1* mutant ferquency. See melting_curves_analysis/melting_analysis_example.R