FRT-PCR Protocol

1. Starting material: A 96-well microtiter plate containing ~ 30 µl genomic ES cell DNA.
2. Add 40 µl 5 mM Tris/HCl pH 8.0 to each well.
3. Merge 1:2 diluted DNA of 4 x 96-well plates to 1 x 384-well plate by transferring 5 µl of diluted DNA according to the following pipetting scheme:

384 well A01 corresponds to 1st 96 well A01

384 well A02 corresponds to 2nd 96 well A01

384 well B01 corresponds to 3rd 96 well A01

384 well B02 corresponds to 4th 96 well A01

…

4. Continue to FRT-PCR:

Component	Amount (µl)
Genomic DNA	5,00
Sterilised, destilled water	2,12
10x reaction buffer (incl. 1,5 mM Mg^{2+})	1,00
2 mM dNTP mix	1,00
Sense primer 2 (10 pmol/µl)	0,40
Antisense primer 4 (10 pmol/µl)	0,40
Eppendorf Taq Polymerase (5U/µl)	0,08
Final volume	10,00

Add 5 µl of PCR mix to each 384 well.

Program: 94°C/120sec + 34x(96°C/15sec; 55°C/15sec; 72°C/60sec) + 72° C/7min

5. Analyse 2 µl of amplfication product on 1,4% agarose gel or proceed directly to sequencing.

6. BigDye (v3) Terminator Sequencing PCR:

Component	Amount (µl)
FRT-PCR product	1,00
Sterilised, destilled water	7,00
Seq. primer 1 (10 pmol/µl)	1,00
Big Dye Terminator Mix	1,00
Final volume	10,00

Program: 96°C/60sec + 25x(96°C/10sec; 56°C/5sec; 70°C/240sec)

7. Primers

Number	**Sequence (5'-> 3')**	**Primer type**	**Tm value**
2	CGAGTGAGGGGTTGTGCTAG	FRT Fwd	61°C
4	CCTATAGTGAGTCGTATTCTCCCG	FRT Rev	63°C
1	CACTATGCGCACAGCTGGTCG	Seq. Fwd	62°C