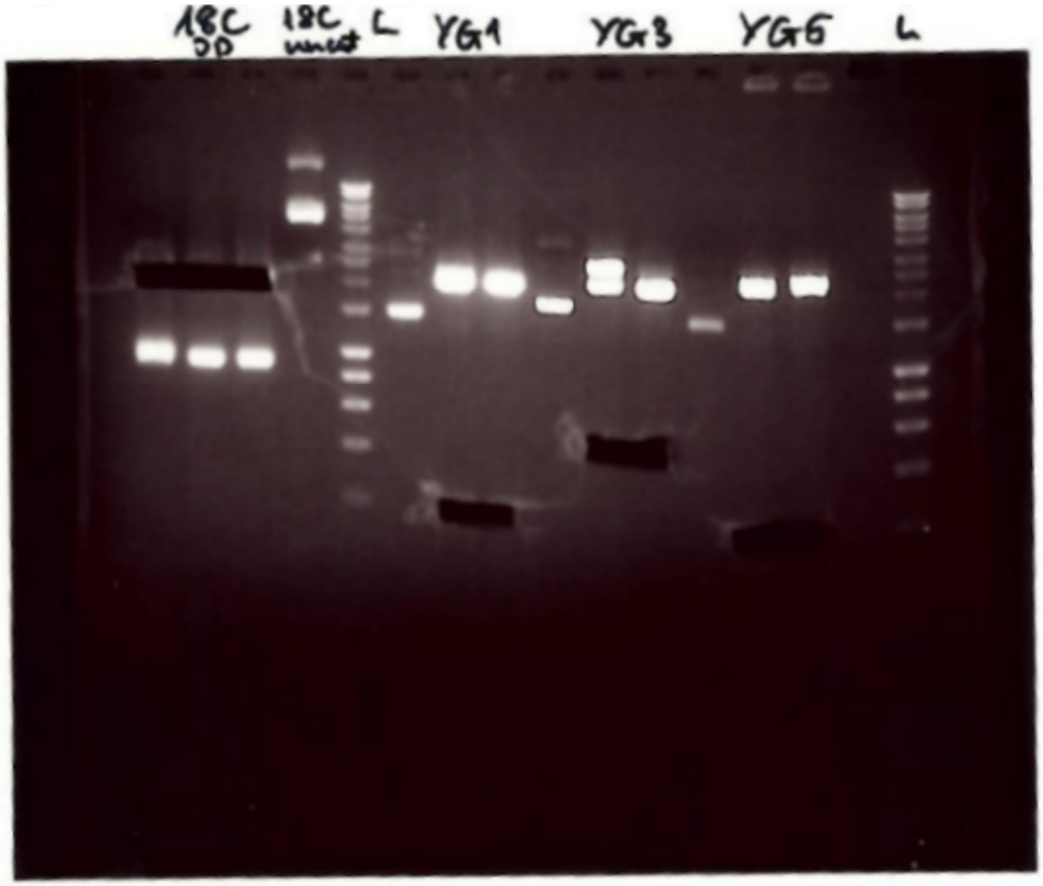
9/9/2013

Aim: Digestion and gel extraction of YG1, YG3, YG5 and 18/C.

Strain: E. coli DH5α

Plasmid: Bba_J61002, pSB1C3

	4	1, 2, 3	6	7, 8	9	10, 11	12	13, 14
	18/C Control	**18/C SpeI PstI**	**YG1 Control**	**YG1 PstI XbaI**	**YG3 Control**	**YG3 PstI XbaI**	**YG5 Control**	**YG5 PstI XbaI**
Water	17	13	17	12.5	17	13.5	15	2
Buffer	2	2	2	2	2	2	2	2
DNA	1	3	1	3.5	1	2.5	3	14
Enzyme1	-	1	-	1	-	1	-	1
Enzyme2	-	1	-	1	-	1	-	1
All values are in µL.		x3		x2		x2		x2



Double digestions and gel extractions of 18C, YG1, YG3, YG5

1% agarose gel; the gel was run for 47 min at 100 V; 1 kb ladder; Green Loading Buffer (Thermo Scientific);

Results:

The 18/C samples were completely digested and the bands around 2 kb containing the vector with the promoter were cut and extracted. The bands for the YG1, YG3 and YG5 peptide genes were as expected, YG3 being twice the size of the other two. The bands around 200 bp (for YG1 and YG5) and 400 bp (for YG3) were cut and extracted.

Next:

Ligation of the plasmid backbone and promoter from 18/C with the YG1, YG2 and YG3 peptide genes.

NSP#

9/9/2013

Aim: Ligation of the plasmid backbone and promoter from 18/C with the YG1, YG2 and YG3 peptide genes.

Strain: E. coli DH5α

Plasmid: Bba_J61002

Formula for calculation of the amount of insert in the ligation reaction:

Amount of vector [ng] * Desired ration * insert [bp] / vector [bp] = Amount of insert [ng]

	18C+YG1	18C+YG3	18C+YG5
Water	11	14	14
T4 Buffer	2	2	2
T4 Ligase	1*	1*	1*
Insert DNA	5	2	2
Vector DNA	1	1	1

All values are in µL. The T4 Ligase was diluted concentration used is 40% of the stock concentration.

NSP#

9/9/2013

Aim: Transformation of the 18C+YG1, 18C+YG3 and 18C+YG5 ligation products in both E. coli DH5α and Wild type K12. Make one control for each strain of bacteria without the ligation products.

Strain: E. coli DH5α, Wild type K12

Plasmid: Bba_J61002

Results:

The 6 transformation plates are growing; the 2 controls are clean.

Next:

Perform a colony PCR in order to select the colonies with the right constructs.

NSP#

10/9/2013

Aim: Performing a colony PCR in order to amplify and verify that we have successfully transformed E. coli DH5α and K12 wild type with 18C+YG1, 18C+YG3 and 18C+YG5 ligation products.

Strain: E. coli DH5α, K12 wild type

Plasmid: Bba_J61002

Master mix:

	600 µL total volume
Nuclease- free water	375.70
5x GoTaq flexy buffer	130
$MgCl_2$, 25 nM	52
PCR dNTP, 10 µM each	13
Forward primer, 25 µM	13
Reverse primer, 25 µM	13
GoTaq DNA Polymerase	3.3

All values are in µL. Sample volume is 20 µL.

Programing of the thermocycler:

Cycle 1 → 95°C / 5 min (Initial Denaturation)

Cycle 2

Step 1→ 95°C / 30 sec (Denaturation)

Step 2→ 58°C / 1 min (Annealing)

Step 3→ 72°C / 1 min (Extension)

Cycle 3 → 72°C / 5 min (Final Extension)

Samples:

1 to 5 → DH5α 18C+YG1

6 to 10 → DH5α 18C+YG3

11 to 15 → DH5α 18C+YG5

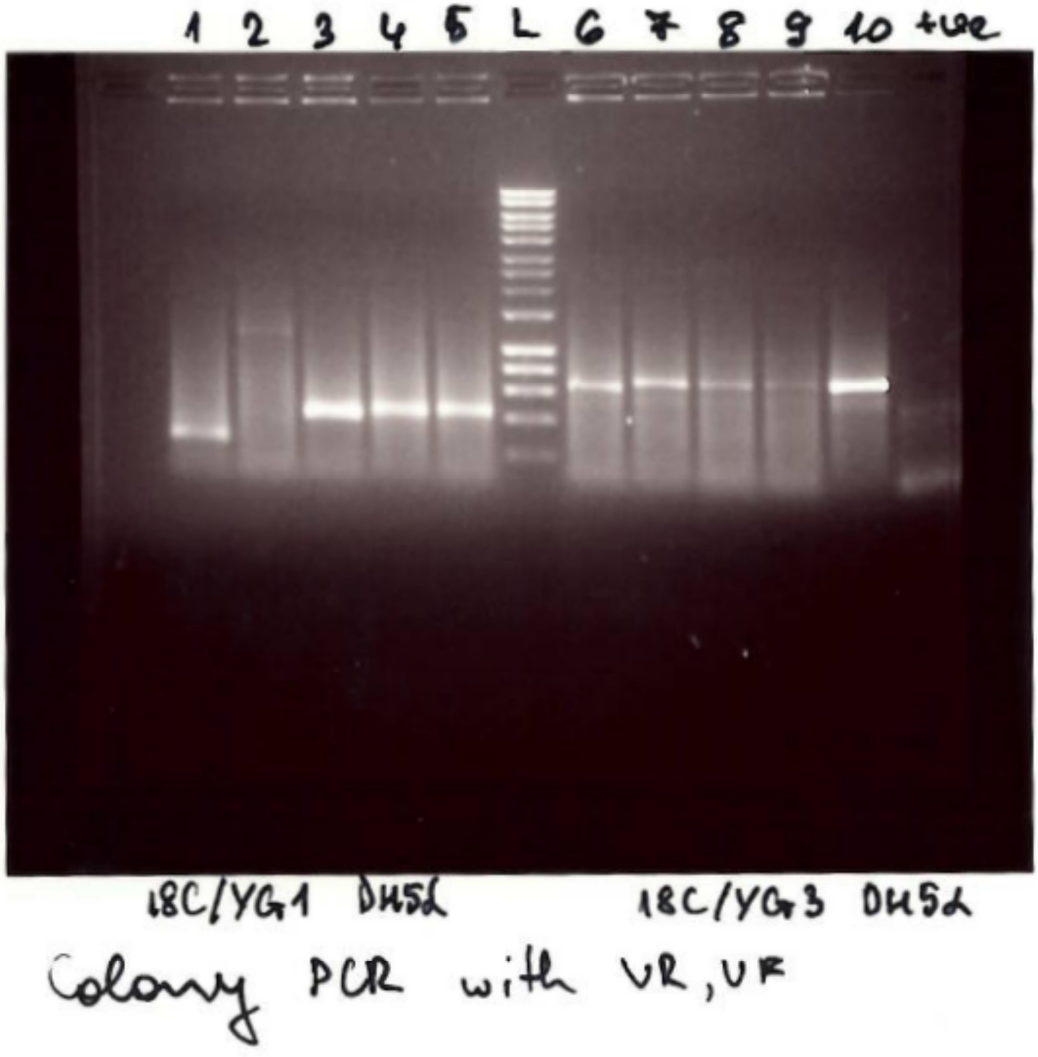
16 to 20 → K12 wild type 18C+YG1

21 to 25 → K12 wild type 18C+YG3

26 to 30 → K12 wild type 18C+YG5

31, 32 → +ve control 18C

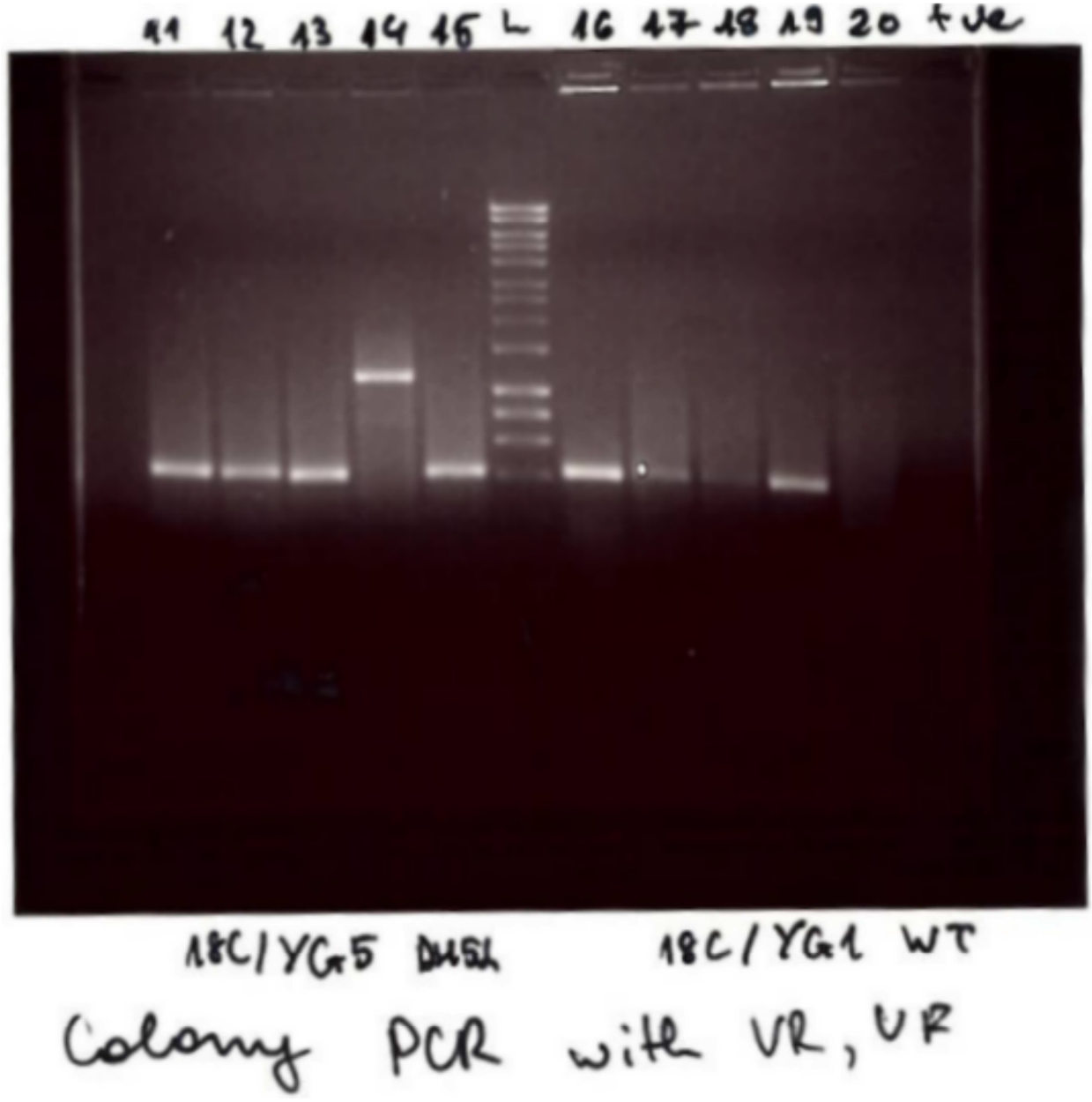
3 µL ladder; 5 µL control; 10 µL PCR products



1% agarose gel; the gel was run for 53 min at 100 V; 1 kb ladder; Green Loading Buffer (Thermo Scientific);

Results:

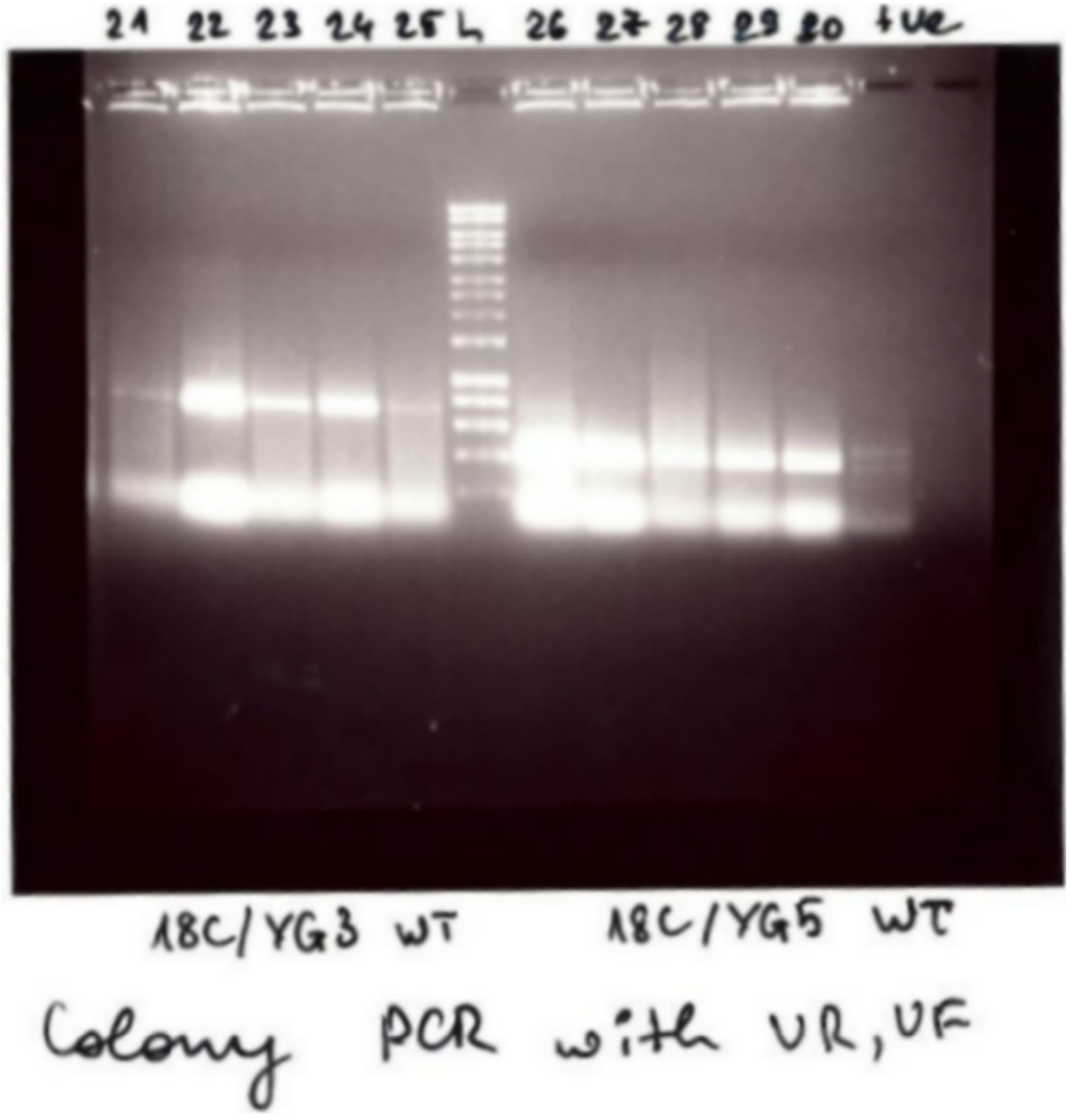
Samples 3, 4, and 5 for DH5α 18C+YG1 show bands just above 400 bp, which is expected since the gene amplified by the VR and VF primers is about 200 bp and they are around 100 bp each. Samples 1 and 2 might not be correct. Sample 2 could have the RFP gene amplified by the primers instead of the YG1. Digestions with SanDI have to be made for further verification of the samples. Samples 6 to 10 for DH5α 18C+YG3 have visible band around 600 bp, which again seems to be the correct construct (400 bp for YG3 + 200 for VR and VF).



1% agarose gel; the gel was run for 55 min at 100 V; 1 kb ladder; Green Loading Buffer (Thermo Scientific);

Results:

Samples 11, 12, 13 and 15 for DH5α 18C+YG5 have bands around 400 bp, therefore they might have the correct construct. Sample 14 has a clear band above 1 kb, which suggests that this is the wrong construct containing the RFP gene. Samples 16 to 19 for K12 wild type 18C+YG1 look like they have the correct construct judging by the 400 bp bands. Sample 20 contains a very faint band that could also be correct.



1% agarose gel; the gel was run for 58 min at 100 V; 1 kb ladder; Green Loading Buffer (Thermo Scientific);

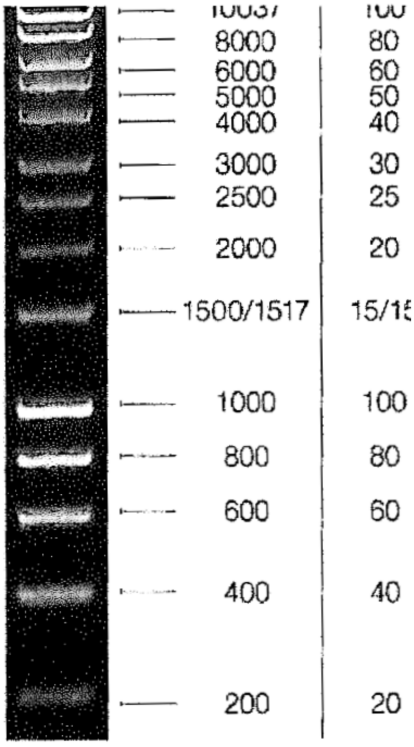
Results:

The gel is not very clear and the reason for this might be the higher concentration of VR and VF primers used for the PCR reaction of these 10 samples. The aggregation of primers might explain the huge bands around 200 bp. Nevertheless, samples 21 to 25 K12 wild type 18C+YG3 look like they

have the right construct with visible bands just above 600 bp. Samples 26 to 30 K12 wild type 18C+YG5 also show the correct band pattern with bands around 400 bp.

Next:

Growing liquid LB cultures of 2, 3, 10, 14, 15, 16, 22 and 26; purifying them and digesting them with SanDI in order to verify the right constructs. Only the YG inserts contain a cutting site for this restriction enzyme.



NSP#

11/9/2013

Aim: Performing single digestions with SanDI and verifying that we have successfully transformed E. coli DH5α and K12 wild type with 18C+YG1, 18C+YG3 and 18C+YG5 ligation products.

Strain: E. coli DH5α, K12 wild type

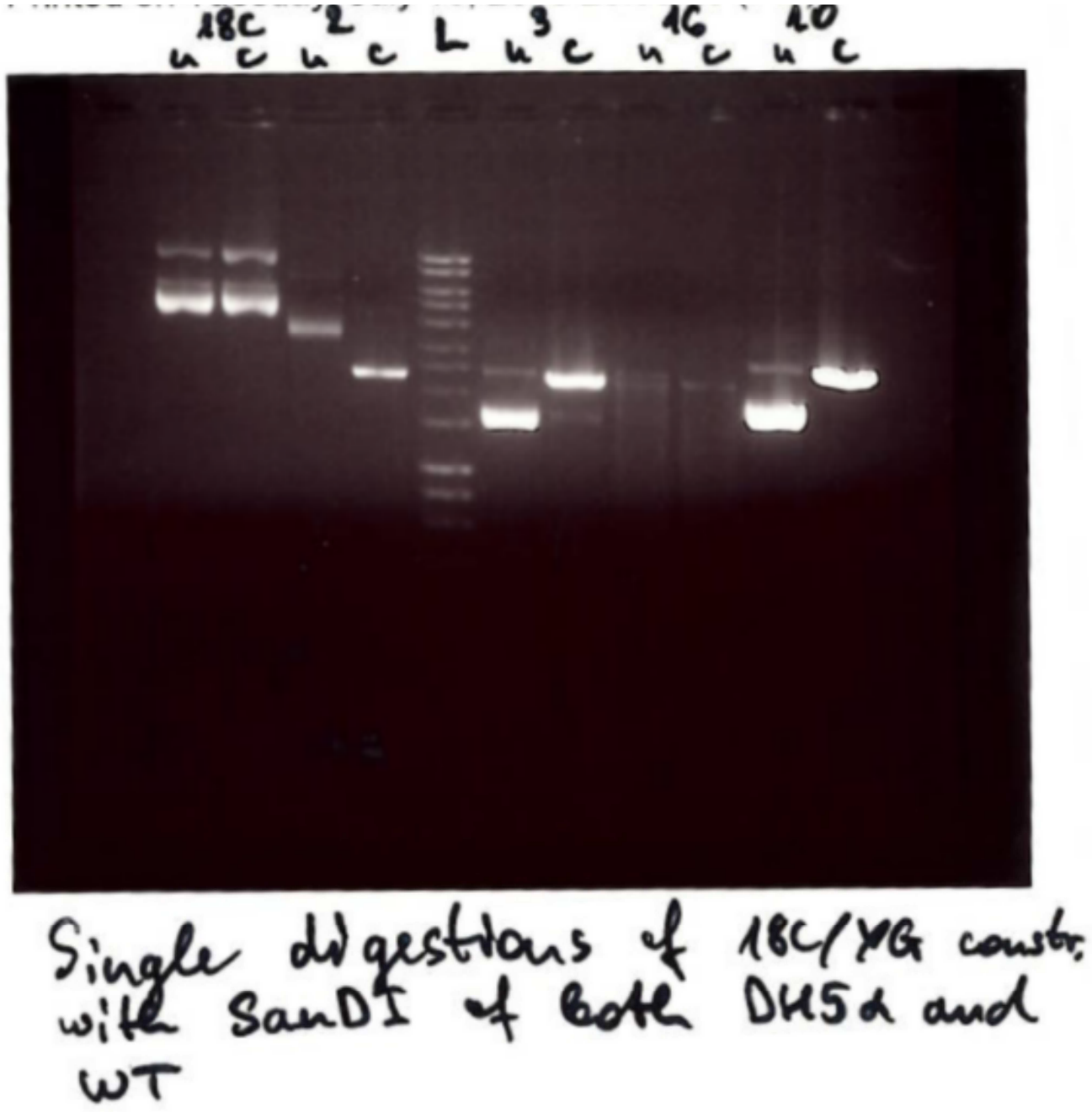
Plasmid: Bba_J61002

	18C Control	18C Cut	Cult. 2 Control	Cult. 2 Cut	Cult. 3 Control	Cult. 3 Cut	Cult. 10 Control	Cult. 10 Cut	Cult. 14 Control
Water	16.5	15.5	13.5	12.5	15	14	14	13	16
Buffer	2	2	2	2	2	2	2	2	2
DNA	1.5	1.5	4.5	4.5	3	3	4	4	2
SanDI	-	1	-	1	-	1	-	1	-

All values are in µL. All samples contain 500 ng of plasmid DNA.

	Cult. 14 Cut	Cult. 15 Control	Cult. 15 Cut	Cult. 16 Control	Cult. 16 Cut	Cult. 22 Control	Cult. 22 Cut	Cult. 26 Control	Cult. 26 Cut
Water	15	15	14	17	16	15.5	14.5	15.5	14.5
Buffer	2	2	2	2	2	2	2	2	2
DNA	2	2	2	1	1	2.5	2.5	2.5	2.5
SanDI	1	-	1	-	1	-	1	-	1

All values are in µL. All samples contain 500 ng of plasmid DNA.



1% agarose gel; the gel was run for 57 min at 100 V; 1 kb ladder; Green Loading Buffer (Thermo Scientific);

1% agarose gel; the gel was run for 57 min at 100 V; 1 kb ladder; Green Loading Buffer (Thermo Scientific);

Results:

18C cannot be cut by SanDI so not surprisingly the digestion patterns of both the cut and uncut samples look similar. Culture 2 has an uncut band between 3 kb and 4 kb but when it is cut there is a clear band just under 2.5 kb. Cultures 3, 10 and 15 have very intense bands and similar digestion pattern. Cultures 16 and 26 have similar bands to the ones from cultures 3, 10 and 15 but the bands of the former are very faint, which suggests that there was unequal amount of DNA in the digestion tubes. The samples from culture 14 (both cut and uncut) have similar bands to the ones from 18C, which indicates that the presence of the RFP gene instead of the YG3 (Culture 14 also had a red colour, so the outcome does not come as a surprise). Culture 22 has a very strange digestion

pattern, since there are bands around 5 kb and 6 kb for the uncut and around 2.5 kb for the cut. This is rather similar to the results for culture 2.

The samples from cultures 3, 10 and 15, which are from DH5α are much more intense compared to the ones from K12 wild type. Cultures 3, 10 and 15 clearly contain the 18C+YG1, 18C+YG3 and 18C+YG5 ligation products.