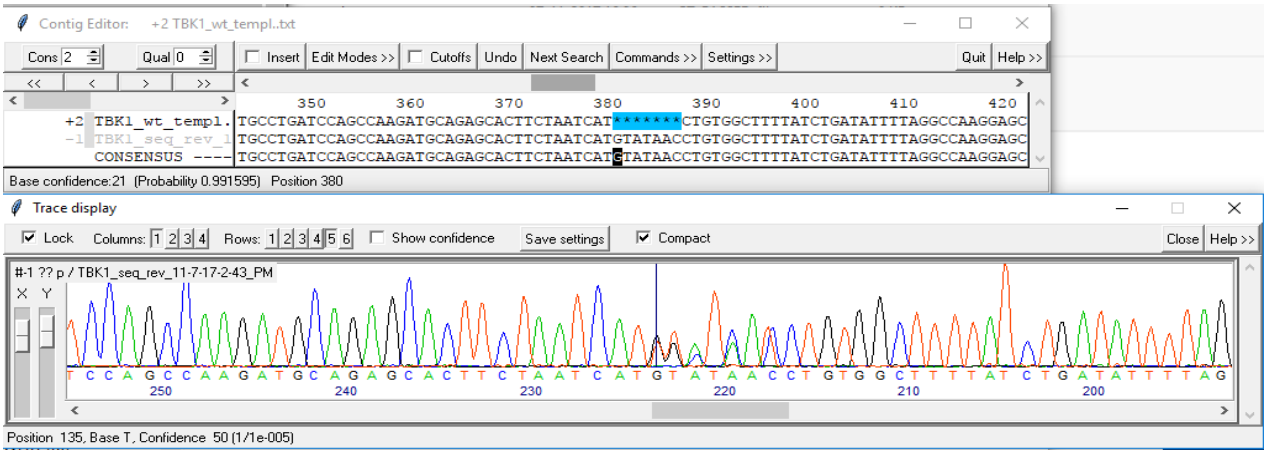
Sequencing analysis:

Screening Primers (923 bp. approx. 385 and 538 bp after digestion with BclI)

TBK1ex2_fw: gagttaagcacagaaagtgatattg

TBK1ex2_rv: ttactccaatctactttgtaggatg



Sequencing of TBK1 KO cl. 24-54, with insert of TGATCAC (cut by BclI) and GTCTAAT (not cut by BclI) in each allele, both resulting in a premature stop codon and an out of frame mutation

Clonal purity assessment:

TBK1_abs: AGAGCACTTCTAATCATCTGTGG

TBK1_abs: GGGATCAAAATCACCAAGTCACA

Product 183

TBK1_abs: TGTTTCTTAGCTGTGTTACTCCC

TBK1_abs: TCAGATAAAAGCCACAGATGATT

Product 127

Alternatively, the following primer pair can be used:

TBK1_abs: TCATGTCAGTAACAGATAATGGGTG

TBK1_abs: TCAGATAAAAGCCACAGATGATT

Product 183

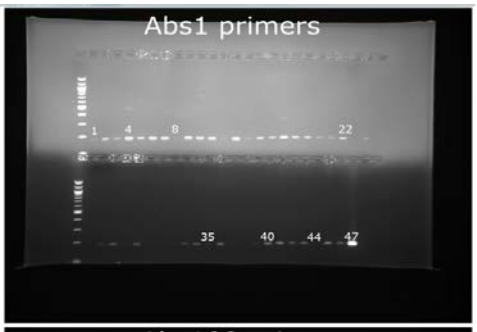
TBK1_abs: TGCAGAGCACTTCTAATCATCTG

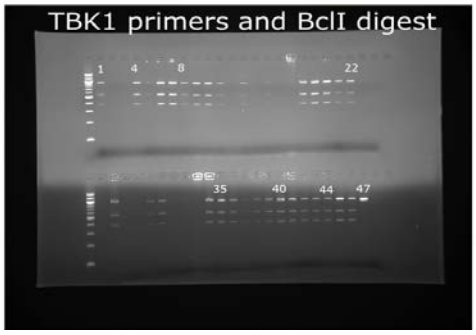
TBK1_abs ATGCAGAGCACTTCTAATCATCT

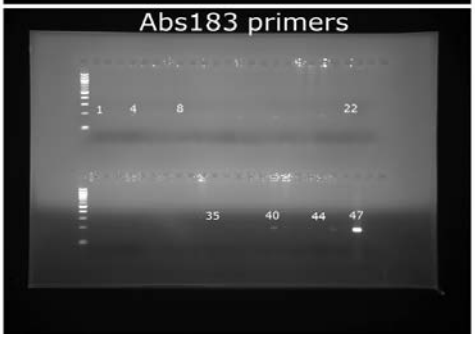
PCR analysis of 48 new single cell clones from TBK1 cl. 24-54

As expected, TBK1 primers produced a heterozygous pattern in all the clones. Abs1 again showed a clear band in almost all the clones, whereas, Abs183 showed a weak band in a few of the clones.

7 ko and 1 wt clone have been selected and are currently being sequenced to confirm purity. If all 7 ko clones seem OK, we will bank the original 24-54 clone.

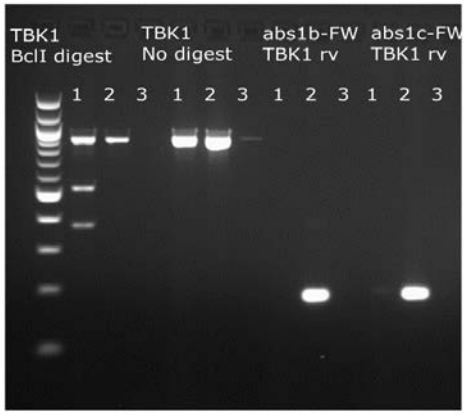






5

PCR with new abs primers



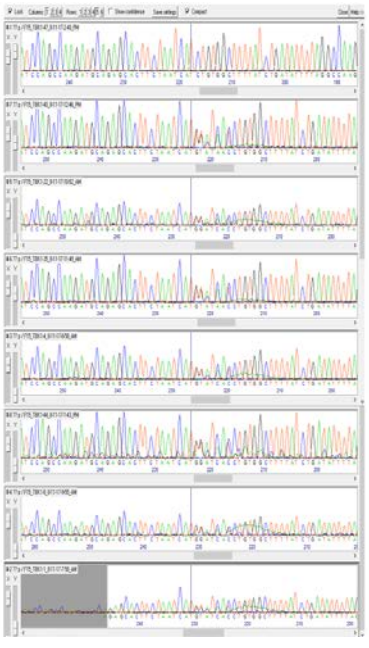
1= TBK1 KO cl. 24-54
2= wt BIONi010-C
3=H2O

No bands are seen with the new abs primers, so the cells will be banked thawed for characterization next week.

7

Sequencing of 1 wt and 7 new single cell clones from TBK1 cl. 24-54

All 7 TBK1 single cell clones have identical sequence at the insertion site. They also contains SNP's further down the sequence corresponding to the WT sequence, which indicates that futher KO's of the alleles have not ocurred.



6