PCR for typing TNFa-ko (Taconic, Ludwig Inst)
Adjusted from Jackson protocol for Tnf^{tm1Gkl}, Standard PCR, Version 2
But CAVE! Ko PCR does not work for the Taconic TNF strain → do Neo (Flt3 mice) PCR!
                                                                        April 11, 2014

Tested for GoTaq-Polymerase and –buffer (Promega)

Primer: Box II
TNFa-comF   **TAG CCA GGA GGG AGA ACA GA** Microsynth 933995
TNFa-wtR   **AGT GCC TCT TCT GCC AGT TC** Microsynth 933996
~~**TNFa-mutR**   **CGT TGG CTA CCC GTG ATA TT**   **(does not work for Taconic strain!)**~~

Isolation of DNA: Quiagen protocol

samples: #   2d2xTNF   276-279

controls: #   wt DNA : NG2 563 and 685

PCR reaction
Program #:   94°C for 3 min; (94°C 30s, 62°C 60s, 72°C 60s) 35 cycles;
                   final elongation 72°C 2min, hold 10°C
instead of this cycle, the cycle of 2d2 can be used

2 ul	DNA	8x
	enzyme mix :	
2.5ul	TNFa-comF (10uM)	20
2.5ul	TNFa-wtR (10uM)	20
2.5ul	TNFa-mutR(10uM)	20
5 ul	5 x TaqPol buffer (GoTaq)	40
0.5 ul	dNTP's 10 mM each	4
2 ul	MgCl$_2$ 25mM	16
0.125ul	Taq polymerase (GoTaq)	2
7.875 ul	water	63
25 ul		
		23 ul / tube

Agarose Gel 1.5%
 bands: WT = 183 bp
        KO = 318 bp