

Project Time	Monday, December 01, 2014			Tuesday, December 02, 2014		
	MSP	AR	ladder	MSP	AR	ladder
9-10			- Phosphorylation of oligos	- Dephosphorylation of parent vector pCHOKE-B	- Dephosphorylation of parent vector pCHOKE-B, filling in 5'-overhangs with Klenow polymerase	- Dephosphorylation of parent vector pOTB7-PC5
10-11	- PCR of gateway cassette		- Annealing of oligos			
11-12 12-13				- Gel isolation of parent vector and XhoI-cleaved PCR product	- Gel isolation of parent vector and insert	- Gel isolation of parent vector
13-14	- Check PCR result on gel					
14-15						
15-16	- Digestion of PCR product and parent vector pCHOKE-B (#1152) with XhoI o/n	- Digestion of cDNA vector (pSG5-rAR, #1752) with BglIII and XbaI o/n; digestion of parent vector pCHOKE-B (#1152) with EcoRV and PmeI o/n	- Digestion of parent vector pOTB7-PC5 (#1461) with BglI and XhoI o/n	- Ligation and transformation into DB3.1 E.coli, plating on ampicillin+chloramphenicol plates	- Ligation and transformation into DH5 $\alpha$ E.coli, plating on ampicillin plates	- Ligation and transformation into DH5 $\alpha$ E.coli, plating on chloramphenicol plates
16-17 17-18 18-19						

**Projects:**      *MSP*                      Denis Belitskin, Shishir Mani Pant  
                          *AR*                              Khalid Saeed  
                          *ladder*                              Girma Raba

**Location:**      Biomedicum Helsinki. Fifth floor, A wing, room A509a2

Project Time	Wednesday, December 03, 2014			Thursday, December 04, 2014		
	MSP	AR	ladder	MSP	AR	ladder
9-10				- Minipreps	- Minipreps	- Minipreps
10-11				- Restriction analysis of minipreps (including one linearization)	- Restriction analysis of minipreps	- Restriction analysis of minipreps
11-12				- Confirmation by sequencing the junctions	- Confirmation by sequencing the junctions	- Confirmation by sequencing the junctions
12-13						
13-14				- Optional: Purification of linearized miniprep (for Gateway recombination)		
14-15						- Digestion of a good clone (= new parent vector pOTB7-BglI2x) and the insert vector (pGEM-3Zf+-Quek2) with SpeI and HindIII o/n
15-16	- Picking clones for miniprep	- Picking clones for miniprep	- Picking clones for miniprep	- Gateway recombination of pENTR-MSP with pCHOKE-DEST and transformation into E.coli		
16-17						

Project Time	Friday, December 05, 2014			Saturday	Sunday, December 07, 2014
	MSP	AR	ladder		
9-10	- Checking results of Gateway re-combination		- Dephosphorylation of parent vector pOTB7-BglII2x (new miniprep from Thursday), gel isolation of parent vector and insert		
10-11					
11-12					
12-13					
13-14					
14-15			- Ligation and transformation into DH5α E.coli, incubation of bacterial plates at 27°C		
15-16	- Checking sequencing results	- Checking sequencing results			
16-17					<b>MSP:</b> Picking clones of Gateway re-combination, <b>ladder:</b> Picking of clones

Project Time	Monday, December 08, 2014			Tuesday, December 09, 2014		
	MSP	AR	ladder	MSP	AR	ladder
9-10	- Minipreps of pCHOKE-DEST-MSP		- Minipreps of pOTB7-BglII2x-insert	-Changing medium on transfected CHO cells	-Changing medium on transfected 293T cells	- Dephosphorylation of parent vector pOTB7-BglII2x-insert
10-11	- Restriction analysis of minipreps		- Restriction analysis of minipreps			- Gel isolation of parent vector and insert
11-12	- Confirmation by sequencing the junctions		- Confirmation by sequencing the junctions			
12-13						
13-14	- Transfection of CHO cells with pCHOKE-DEST-MSP DNA	- Transfection of 293T cells with pCHOKE-B-rAR DNA	- Digestion of a good clone with NaeIII and BglII o/n			- Run-away ligation and transformation into Xl1Blue E.coli
14-15						
15-16				-Changing medium on transfected 293T/CHO cells to start conditioning in serum-free medium		
16-17						

Project Time	Wednesday, December 10, 2014			Thursday, December 11, 2014		
	MSP	AR	ladder	MSP	AR	ladder
9-10	- Start selection of transfected cells (to generate a stable cell line)			- Harvesting supernatant and PAGE of (2-3x conc.?) sups	- Harvesting cell lysates and PAGE	- Minipreps of final constructs
10-11						- Restriction analysis of minipreps
11-12						
12-13						
13-14				- Western blotting until primary antibody (anti-V5, 1:5000, Invitrogen, o/n)	- Western blotting until primary antibody (anti-hAR, 1:1000, SantaCruz sc-13062, o/n)	
14-15			- Checking results of transformation			
15-16			- Picking many clones (6x4=24)			
16-17						

<b>Friday, December 12, 2014</b>			
<b>Project Time</b>	<b>MSP</b>	<b>AR</b>	<b>ladder</b>
9-10	- Secondary anti-body → ECL with Odyssey	- Secondary anti-body → ECL with Odyssey	
10-11	- Change medium and antibiotic on cells for stable selection		
11-12			
12-13			
13-14			
14-15			
15-16			
16-17			