Protocol for PCR Construction of HPV detector plasmid

1. HPV Detector Plasmid (uncut) linearised with KpnI for 4 hours at 37 degrees & heat inactivated at 80 degrees for 5 minutes

Sample	**Volume (ul)**
DNA (68ng/ul)	1.47 (100ng)
KpnI	0.5
Buffer 4	2
H2O	16.03
Total	20

2. Digestion was diluted 1 in 1000 ul of TE buffer (5pg DNA per ul)
3. PCR reaction was set up as follows:

PCR Reactions :

	1	**2**	**3 (Control)**
DNA (5pg/ul)	4	4	4
Solis Biodyne 5x Hot Start	4	4	4
F Primer	1	1	0
R Primer	1	1	0
H2O	10	10	12
Total	20	20	20

PCR Conditions:

Step	**Temperature (degrees celsius)**	**Time**	**Number of**

			Cycles
Initial denaturation	95	15 mins	1
Denaturation	95	30 sec	35
Annealing	56	40 sec	35
Elongation	72	3 min	35
Final Elongation	72	7 min	1

1. Run 3ul of PCR products on gel to check if present
2. DpnI digest carried out by adding 0.5ul of DpnI to reaction tube with PCR product - incubate for 1 hour @ 37 degrees & 80 degrees for 10 mins
3. PCR clean up carried out after digest
4. Run 5 ul of clean up product on gel
5. Quadruple digest carried out on sample by adding 1 ul of NtBsQI + 5ul Buffer 4 and incubating for 2 hours at 50 degrees. The final 3 enzymes (KpnI, HindIII & NtBsI) were then added and incubation continued for 6 hours at 37 degrees
6. Digested reaction was reacted with decoy oligos by adding 1ul of 100mM decoys (R & L) and allowing to sit for 10 mins at room temperature
7. PCR Cleanup was carried out again.
8. Run 5ul of product on gel to check DNA is present

Testing PCR Constructed Detector

Mixes Yielded

	Sample	**Reaction**
1	PCR product with DpnI digest	A
2	PCR product without DpnI digest	
3	PCR Control (no detector)	
4	PCR product with DpnI digest, PCR cleanup, 4x digest	B & C
5	PCR product with DpnI digest, PCR cleanup, 4x digest, Decoy reaction, PCR cleanup	
6	Digested detector (made 10/6/15)	

7	Digested detector - working (made Nov. 2014)	

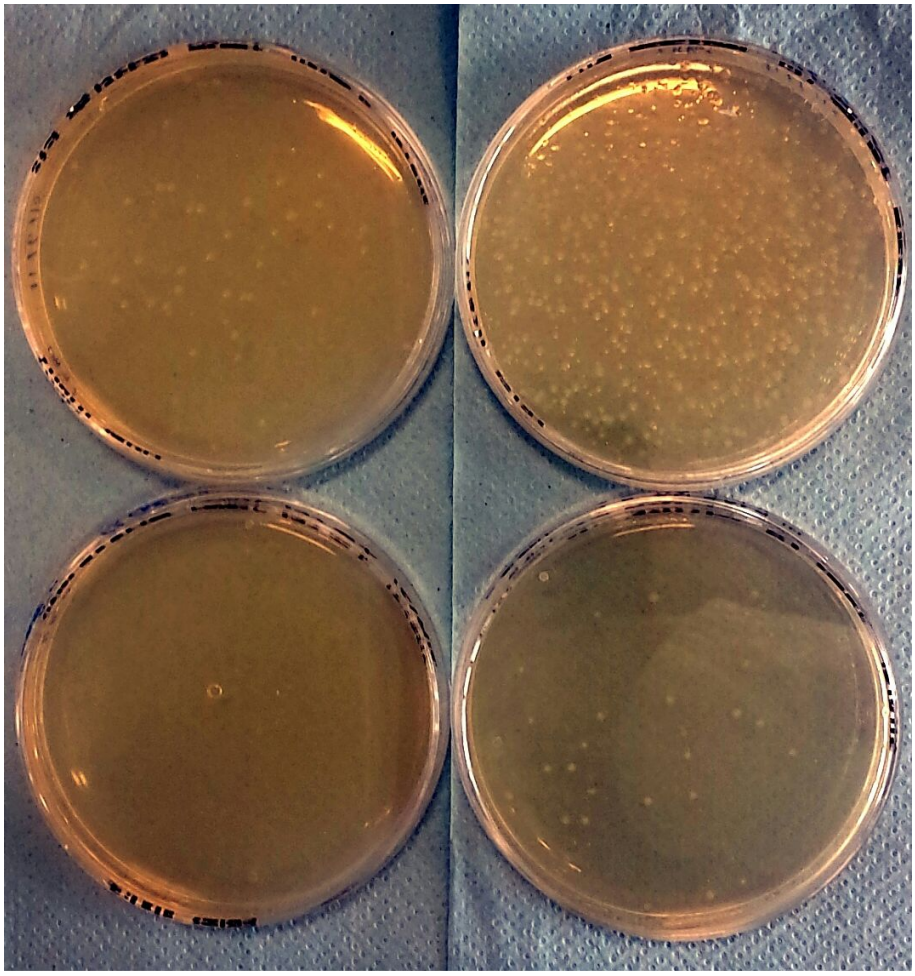
16/6/15 - Reactions carried out prior to transformation:

T	Target - Detector Reaction with Target
C	Control - Detector Reaction without Target
Detector Reaction	**Addition of GFP to Detector mixes 4 & 5** 0.3 ul GFP added per 2ul of detector **Detector Reaction** See other protocol 1ul of Detector + GFP used for each 1 ul of ds target used

Results

- Results of transformation after 5ul of each reaction transformed on 16/6/15

Reaction	Non - GFP (Target)	GFP Expressing CFU
6T	16	38
6C	0	4
4T	0	1
4C	0	6
5T	13	68
5C	0	33



Transformation of cells with Digested Detector (right) and PCR Amplified detector (left). Plates with target on top, no target on bottom. GFP also added to reaction. (16/6/15)

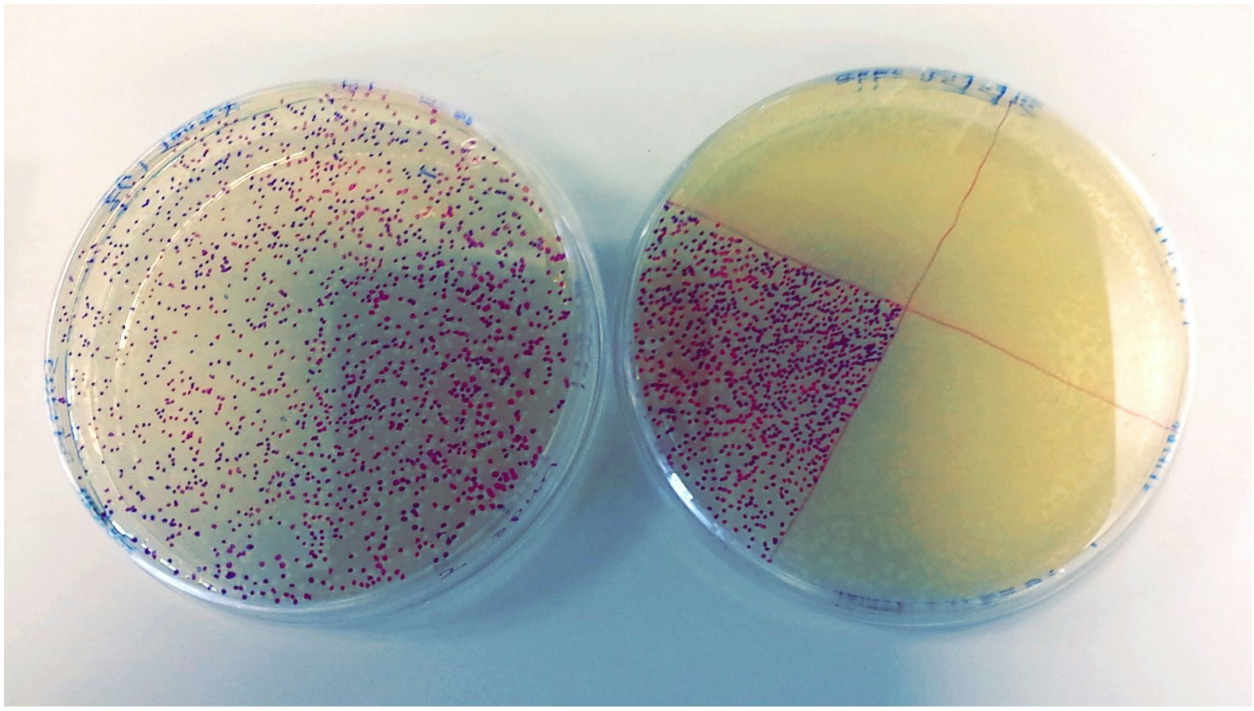
17/6/15 - Repeat of Transformation using Highly Competent Cells and Detectors 5 & 6

- Detector reaction carried out as before
- Transformation followed NEB protocol for highly competent cells

Results of transformation using highly competent cells and detectors 5 & 6 (PCR Mix 5 & Digested detector)

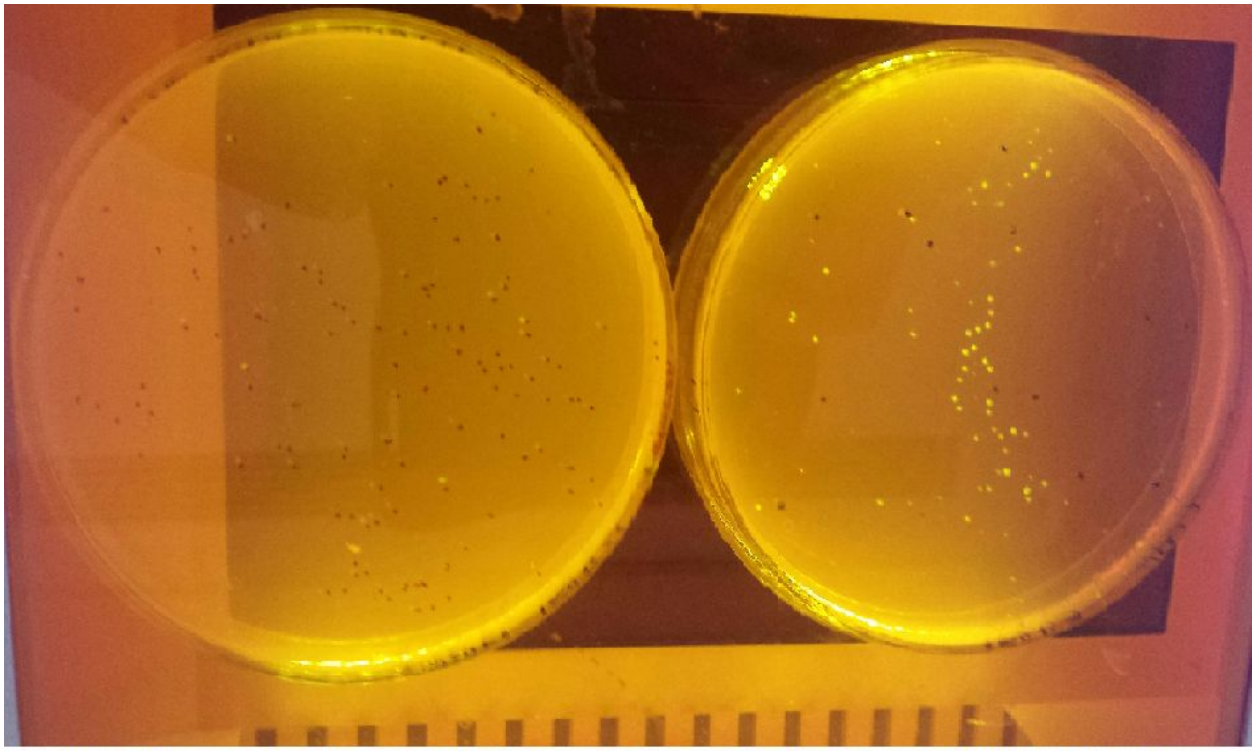
	NGFP	GFP	Ratio
Digested detector + target	2,200	1,950	1.128
PCR 5 + target	761	838	0.908

Digested Detector + no target	11	1,500	0.007
PCR 5 + no target	0	1,500	0

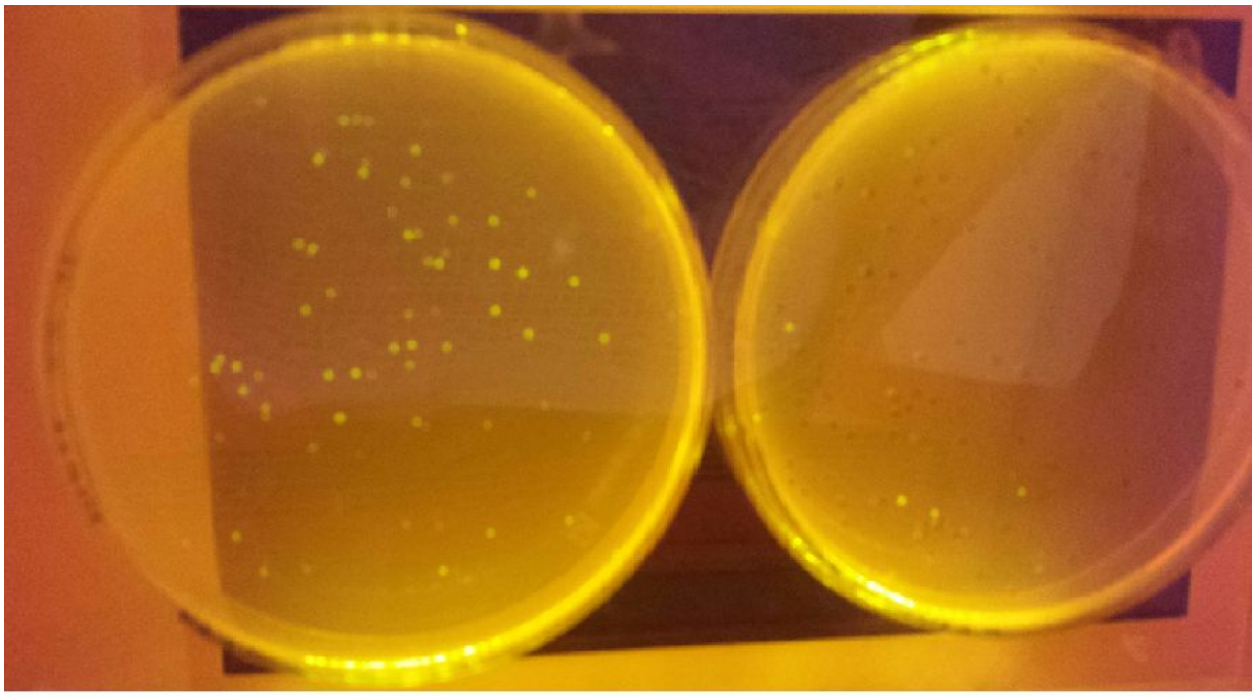


Transformation of highly competent cells with digested detector (right) and PCR amplified detector (left) after reaction with target. Blue points note NGFP containing CFUs (not detector plasmid). (18/6/15)

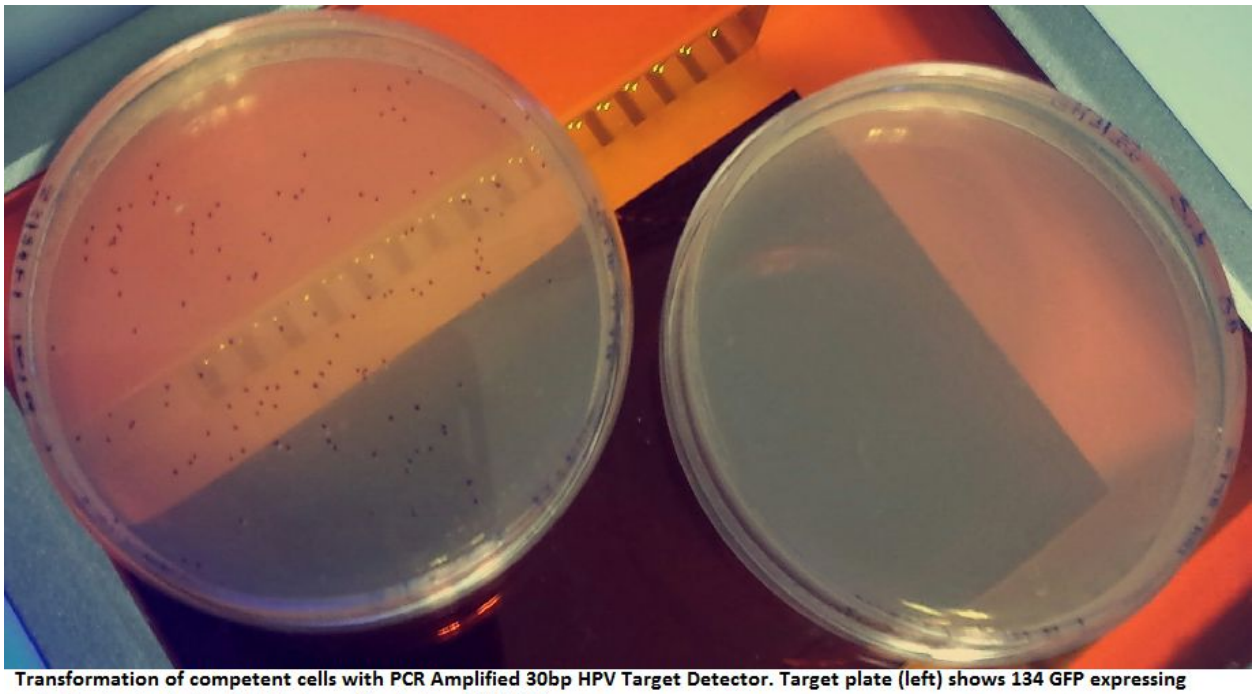
Testing PCR



Transformation of competent cells with 30bp HPV Target Detector (left) and 55bp HPV Target Detector (right). Blue points note target - positive CFUs. (22/6/15)



Transformation of competent cells with PCR Amplified SRY Target Detector. Control plate (left) shows 78 GFP expressing CFUs. Target plate (right) shows 89 NGFP expressing CFUs (detector + target) and 7 GFP CFUs. (22/6/16)



Transformation of competent cells with PCR Amplified 30bp HPV Target Detector. Target plate (left) shows 134 GFP expressing colonies. Control plate (right) shows 0 colonies. (22/6/15)

Results of Transformation using PCR detectors (22/6/15)

	GFP	NGFP
HPV PCR Amplified	8	0
HPV PCR Amplified + Target	188	12
HPV-GFP PCR Amp	0	0

HPV-GFP PCR Amp + Target	356	0
HPV-GFP 30bp Target	0	0
HPV-GFP 30bp Target + Target	~550	0
SRY PCR Amp	80	4
SRY PCR Amp + Target	14	178

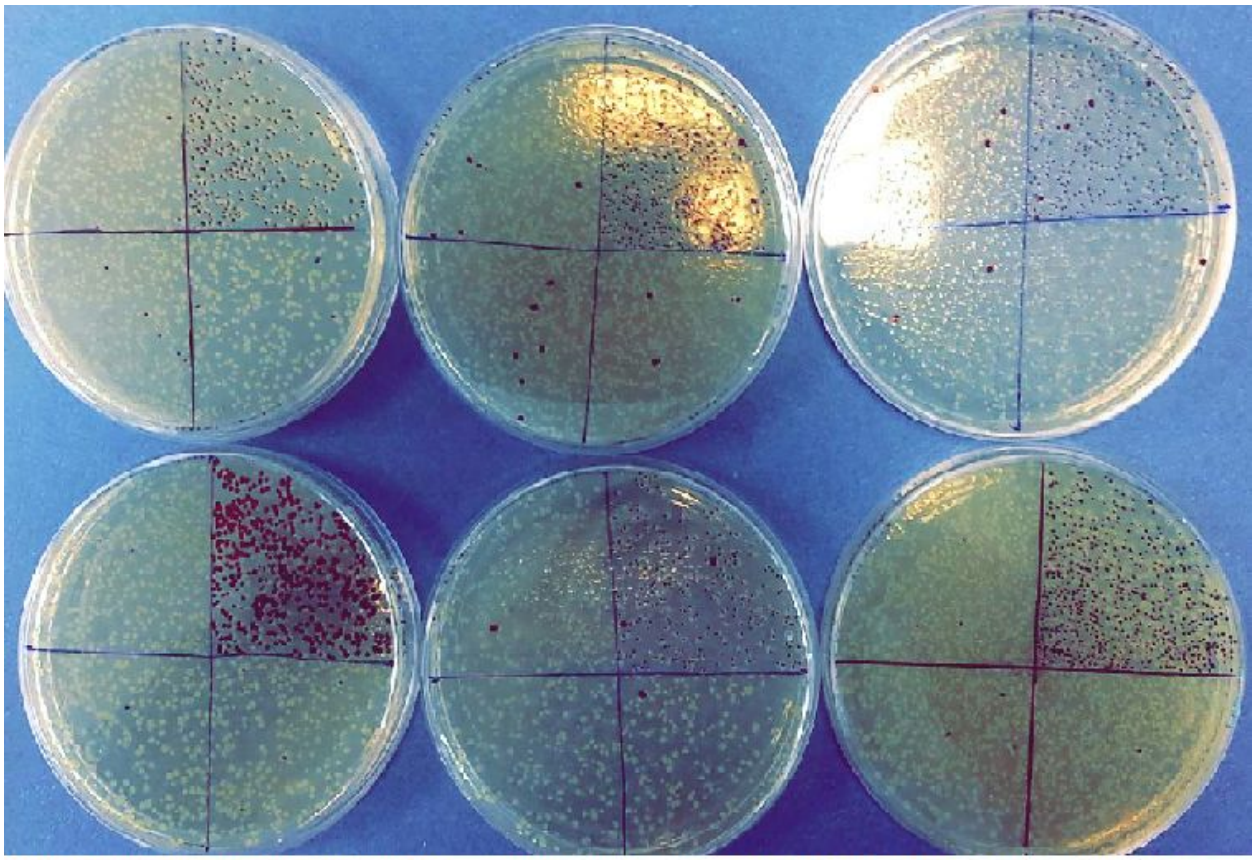
24/6/15 Transformation of SRY Detector with Genomic DNA

Tube	**1 - Male (digest)**	**2 - Male (no digest)**	**3 - Female (digest)**	**4 - No DNA (control)**
SRY Detector	1	1	1	1
GFP (0.01ul plasmid /ul)	1	1	1	1
Tris	1	1	1	1
Male - Digested DNA	1	0	0	0
Male - Undigested DNA	0	1	0	0
Female - Digested DNA	0	0	1	0
H2O	6	6	6	7
Total	10	10	10	10

Results of Transformation (25/6/15) & (26/6/15)

Tube	1 - Male (digest)			2 - Male (no digest)			3 - Female (digest)			4 - No DNA (control)		
NGFP	2	9	15	2	4	17	7	12	7	2	2	
GFP	17	1216	1064	15	856	1840	25	1128	2104	0	1572	
Adjusted Ratio	0.0059	0.007	0.014	0.0067	0.005	0.009	0.014	0.011	0.003	0	0.001	
Average	0.00897			0.0069			0.0093			0.0003		
Average 2	0.0105			0.007			0.007			0.0005		

Note : Test 1 used less Control GFP, Average 2 adjusts for this.



Transformation carried out on 25/6/15 using SRY Detector and genomic samples from male and female mice. Male digested DNA (Left), Male undigested DNA (Middle), Female digested DNA (Right). All samples tested in dublicate.