Experimental Flow

1. Subcloning

a. Gene Preparation

PCR	to replicate DNA purchased in order to increase success chance when inserting GOI
PCR Purification	to produce pure DNA strands
Gel electrophoresis	to confirm amplified gene has the same size as original

b. Ligation

Plasmid digestion	to linearise the plasmid for ligation
PCR product digestion	to produce “sticky ends” on PCR product so that it binds to plasmid
Gel electrophoresis	to confirm the gene has been digested properly
Ligation	to combine the GOI with plasmid backbone
Bacterial transformation + selection	to replicate plasmids for colony PCR

c. Colony PCR

Lyse the bacteria and culture in LB broth	to extract plasmids inside bacteria
PCR Check	to screen for successfully ligated colonies
Plasmid extraction	to obtain plasmids inside successfully ligated colonies

2. Testing gene sequences

- Constitutive promoter, PSMA promoter, Gluc

a. Sequence checking

Construct plasmid	Constitutive promoter: - Promoter: probasin promoter - Protein: GFP PSMA promoter: - Promoter: PSMA promoter - Protein: GFP Gluc: - Promoter: probasin/PSMA promoter - Protein: Gluc
Transfection	to transfer the plasmid into cells 1) PSMA (+) PCa cell line (MLLB-2) 2) PSMA (-) PCa cell line (PNEC 30) 3) Normal prostate cell (YPEN-1)
Gluc assay detection	to obtain results for the experiments to confirm 1) GFP/ Gluc is expressed in every situation 2) Resulting light intensity is proportional to concentration of GFP/ Gluc and cell

3. Polymer synthesis

a. Dual aptamer design (DUP-1, A10-3.2)

Attach PEG on polymer (PAMAM)	to reduce cytotoxicity of product and make it easier to attach aptamers
Attach aptamers	*Aptamers bind to specific substances*
Check aptamer is attached by FRET analysis	*fluorescence resonance energy transfer (FRET) microscopy*
Check aptamer binds to PCa cells	- Prepare polymer + aptamer attached with BODIPY - BODIPY will glow inside cell - Detect using fluorescence inverted microscope
Combining plasmid and polymer	To put the plasmid into the polymer

4. Obtaining results

a. Detection of PCa cells

Synthesise the polymer w/ aptamer & plasmid	Plasmid: probasin promoter/ PSMA promoter + Gluc
Mixing with cell lines	Each of the below with different concentrations + (if possible) mix PCa + normal together 1) PSMA (+) PCa cell line (MLLB-2) 2) PSMA (-) PCa cell line (PNEC 30) 3) Normal prostate cell (YPEN-1)
Gluc assay detection	To detect the intensity of the Gluc produced - Measure intensity at different time periods after the reaction has started - Plot graph of intensity vs time to view when does the reaction peaks/ when does Gluc starts to decompose

b. Killing of PCa cells

Synthesise the polymer w/ aptamer & plasmid	Plasmid: probasin promoter/ PSMA promoter + Gluc + OmpA PD-L1 nanobody
Mixing with cell lines	Each of the below with different concentrations + (if possible) mix PCa + normal together 4) PSMA (+) PCa cell line (MLLB-2) 5) PSMA (-) PCa cell line (PNEC 30) 6) Normal prostate cell (YPEN-1)
OmpA PD-L1 nanobody detection → SDS-page + silver staining	To detect the concentration of the amount of PD-L1 nanobody produced