# Molecular Biology Project Draft

Gene Product Targeting for Breast Cancer

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## Background

This paper explores product targeting methods for breast cancer that have spread to lymph nodes. Through these methodologies it is theoretically possible to pause the extension of Telomeres, whose existence allows the rapid continuous growth of cancer cells. The methods of Telomere length maintenance to be discussed are C-Circles and Telomerase. "C-Circles are a self-overlapping structure at the end of DNA, and Telomerase is a protein product of the hTERT gene, whose over-expression can be a sign of Cancer"[2]. Once the type of Telomere length maintenance is determined (or mix of both), dCas9 can be used to block transcription and thereby halting or slowing down the replication process of cancer cells. Stopping these mechanisms would prevent the extension of Telomeres in the DNA, causing possibly leading cell death upon continuous replication. The strength of this method can then be determined by Spectral Analysis for the absence of Telomerase expression, and a C-Circle Assay (CCA) for the removal of C-Circles.

## Experimental Design

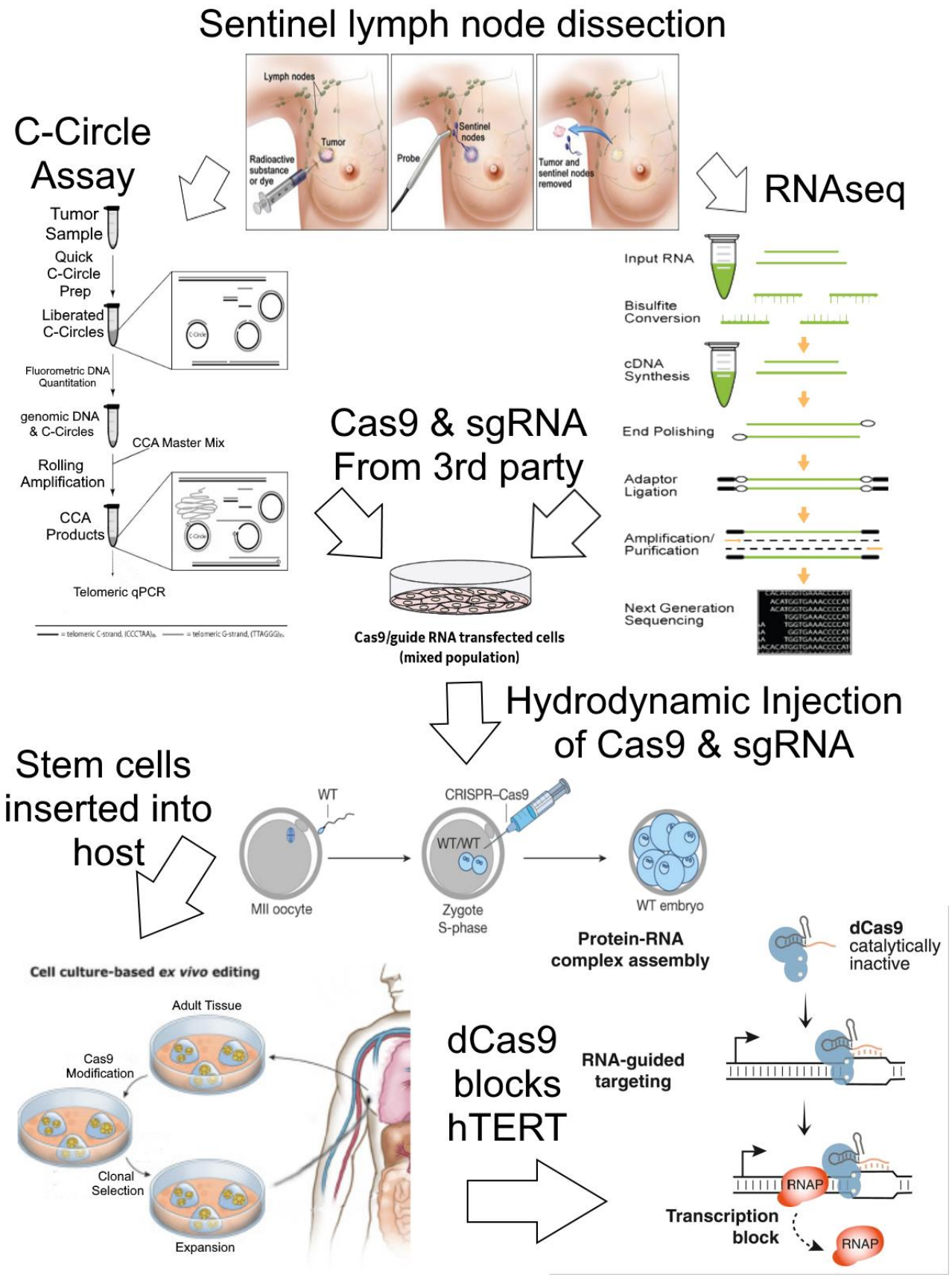
Initially through a Sentinel lymph node dissection (SLND)**,** sample tissue is collected from the patients arm pit to remove the tumor and lymph nodes. Once the tumor DNA is cloned, the sample cells can then be tested to determine their Telomere length maintenance system. To do so, RNAseq will be used due to the small amount of required starting material and little to be known about the sequence. Paired with a reference genome of a healthy lymph node, the expression levels of the hTERT gene can be compared to determine the expression level of the hTERT gene. If an over expression is determined, then Telomerase is known to be used to as a Telomere length maintenance system. Normally, one may use Illumina Deep Sequencing to obtain the sequence of hTERT to be used as the sgRNA in dCas9, but because it is commonly known (5' TGGTTTCTGTGTGGTGTCA 3') and one can simply purchase the sequence or make one their own (not recommended). To test for ALT+ sequences, CCA is to be conducted using the original tumor sample. If a relatively large product remains, then the sequence is ALT+. Similarly to the hTERT gene, the sequence of C-Circles is also commonly known (5' TTAGGG 3' repeat) and can be purchased as a sgRNA sequence via a third party company, if the tested sequences are ALT+. With vital sgRNA acquired it is imperative to store it as a vector with dCas9 by induced homologous recombination for mass production and ease of access. The dCas9 and sgRNA plasmids can then be inserted into stem cells via hydrodynamic injection which in turn are inserted into the tumorous area ex vivo.

## Analysis

Given a period of healing time from the SLND and hydrodynamic injection, it is then possible to analyze the results. To do so, another SLND is to take place on the same arm as the first time. Similarly as before, tests must be conducted to determine if the cell's hTERT gene is expressed and if the C-Circles have been removed, which can be done via RNAseq and qPCR respectively. During RNAseq the cell's RNA is reverse-transcribed, sheared and incorporated into a flow-cell where images are processed by a machine and software as multidimensional matrix. The matrix is then manipulated through a Principal Component Analysis (PCA) to reduce dimensionality, and ran against a series of feature based eigenvectors of similar cDNA sequences. The result is a list of values in order of feature correlation, the first result is the cDNA sequence most similar to the input cDNA. Based on the eigenvalue of the output you can then determine the corresponding features that are absent or present in the sequence. If the eigenvalue is low in the vector for hTERT expression, it is definitive that hTERT has been inhibited. To detect C-Circles, a CCA is to be conducted using the original tumor sample (at least 100 cells)[6]. CCA, similar to PCR, is a process in which the DNA is replicated in a test tube. CCA however, does not use a primer which allows only self-replicating sequences to have products, such as C-Circles. Thereby the amount of product visualized would be a direct correlation to the existence of C-Circles in the genomic sequence.

## Cautions & Limitations

It is imperative to note that dCas9 requires the existence of a PAM sequence near the location of the desired inhibited sequence. Without one, dCas9 will not function properly and the tumorous cells will continue to spread. SLND alone can cause a lot of damage to the patient's nervous system especially when regarding the removal of high level axilla, and has many side-effects such as swelling, infection and axillary web syndrome[22]. It is also possible that due to the lack of Telomerase, tumorous and surrounding cells may encounter mutations or commit mass cell death leading to increasing problems. Due to human cells having another copy of their chromosomes, this method may not fully override mutations in the cancerous cells.


Sentinel lymph node dissection
Lymph nodes
Tumor
Radioactive substance or dye
Sentinel nodes
Probe
Tumor and sentinel nodes removed
C-Circle Assay
Tumor Sample
Quick C-Circle Prep
Liberated C-Circles
Fluorometric DNA Quantitation
genomic DNA & C-Circles
CCA Master Mix
Rolling Amplification
CCA Products
Telomeric qPCR
= telomeric C-strand, (CCCTAA)n
= telomeric G-strand, (TTAGGG)n
RNAseq
Input RNA
Bisulfite Conversion
cDNA Synthesis
End Polishing
Adaptor Ligation
Amplification/ Purification
Next Generation Sequencing
Cas9 & sgRNA From 3rd party
Cas9/guide RNA transfected cells (mixed population)
Hydrodynamic Injection of Cas9 & sgRNA
Stem cells inserted into host
WT
MII oocyte
CRISPR–Cas9
WT/WT
Zygote S-phase
WT embryo
Protein-RNA complex assembly
dCas9 catalytically inactive
Cell culture-based ex vivo editing
Adult Tissue
Cas9 Modification
Clonal Selection
Expansion
dCas9 blocks hTERT
RNA-guided targeting
RNAP
Transcription block
RNAP

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