The IISc Experience

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My Lab

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Department of Molecular Reproduction and Developmental Genetics

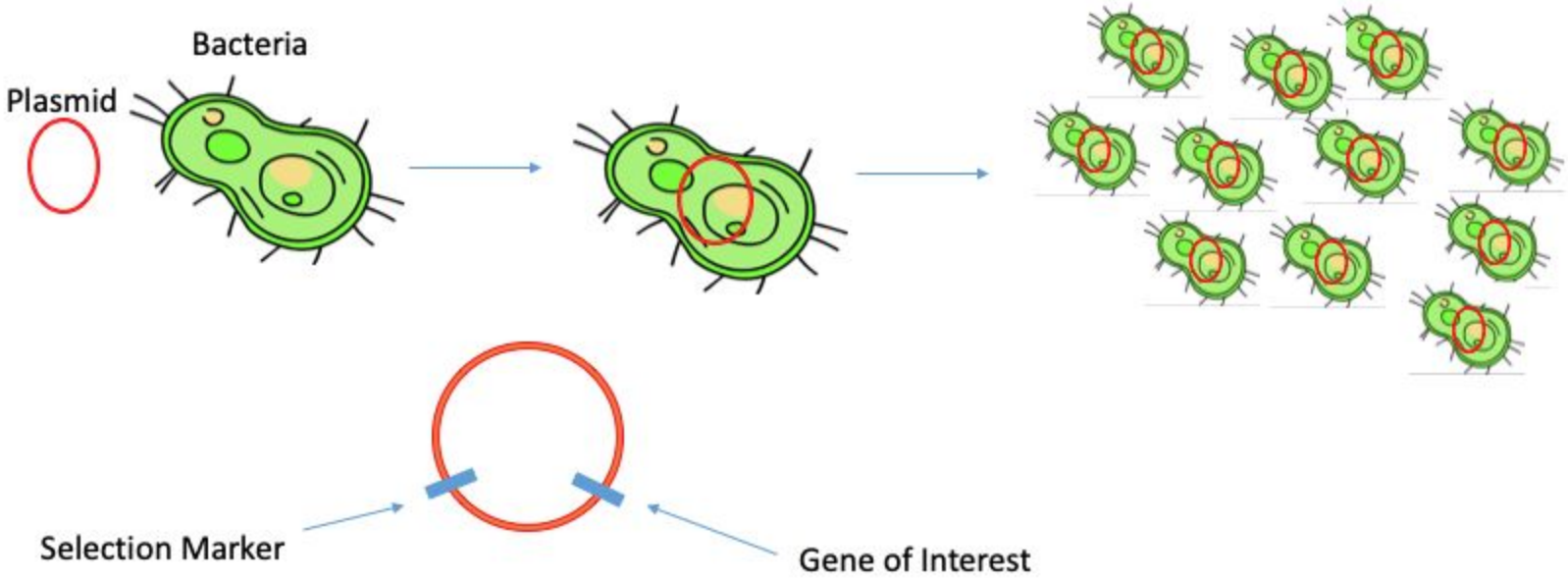
Primarily studies the mechanisms of metastasis

My Internship: Basic Molecular Methods

- Competent cell preparation/transformation
- Plasmid isolation
- RNA isolation
- DNase treatment/-RT PCR
- cDNA Synthesis/+RT PCR

Competent Cell Prep and Transformation: Overall Goal

Insert a plasmid containing a gene of interest into bacteria in order to make more of your gene



Competent Cell Preparation: Calcium Chloride Method

DH5α E-coli cells
1. endA-
2. recA-
3. hsdR17
4. Δ(lac3)M15

Aim: Alter cells so that they can take up exogenous DNA through transformation

Principle: Calcium ions allow the plasmid of interest to interact with lipopolysaccharides on the cell wall

Glycerol Stock of DH5α Cells from company → Snap cool the culture when OD600 indicates logarithmic growth → Calcium Chloride treatment → Glycerol Stock of competent DH5α Cells

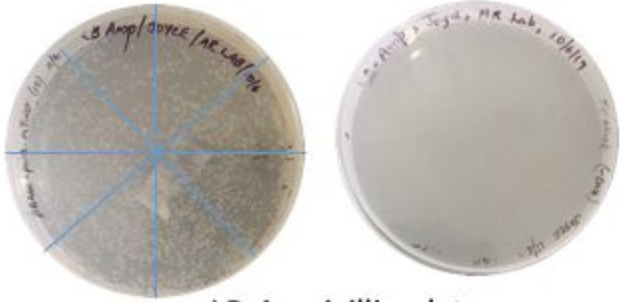
Transformation

Aim: Transform the competent cells with a plasmid coding for pBabe-puro-mTWIST

The plasmid contained an Ampicillin resistance marker to select for cells with successful transformation

With Plasmid

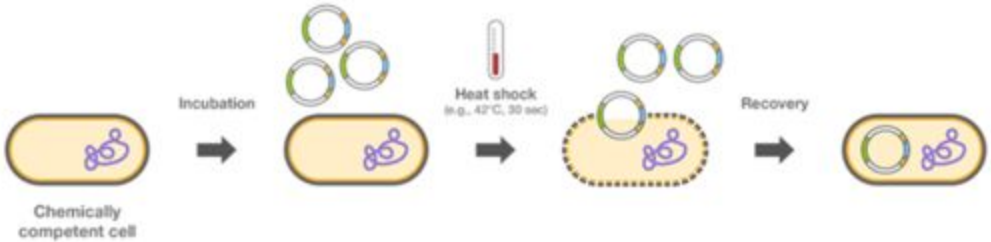
Without Plasmid



LB-Ampicillin plates

Transformation Efficiency Check:

$$\text{Efficiency} = \frac{CFU}{ng\ DNA} = \frac{2640\ colonies}{5\ ng\ DNA} = 528$$



DH5α competent cells → Add plasmid DNA → Heat shock → Select cells with LB-Amp plate

Plasmid Isolation

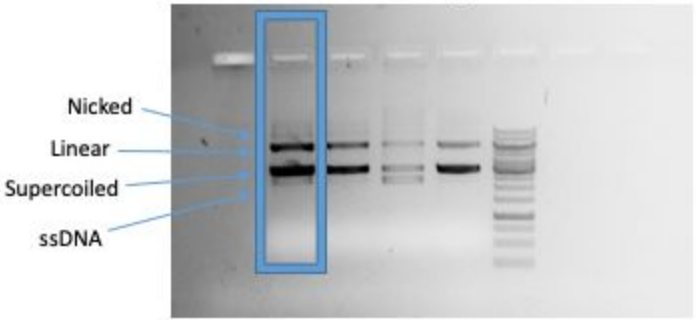
Aim: Remove and purify the amplified plasmid from transformed cells

Principle: Alkaline solution will cause the degradation of RNA and the denaturation of dsDNA. The smaller plasmid renatures faster than the chromosomal DNA, allowing for its selection in the aqueous layer

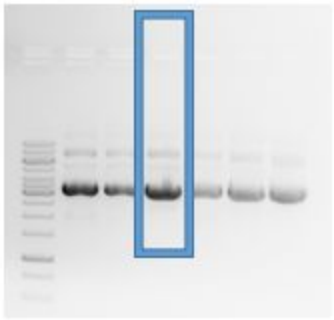
Nanodrop Readings
pBABE-puro-mtwist

Sample	Concentration (ng/µL)	Purity (A260/280)
Alkaline Lysis	1986.9	0.6
Quiagen Mini-Prep	151.8	1.94

Alkaline Lysis

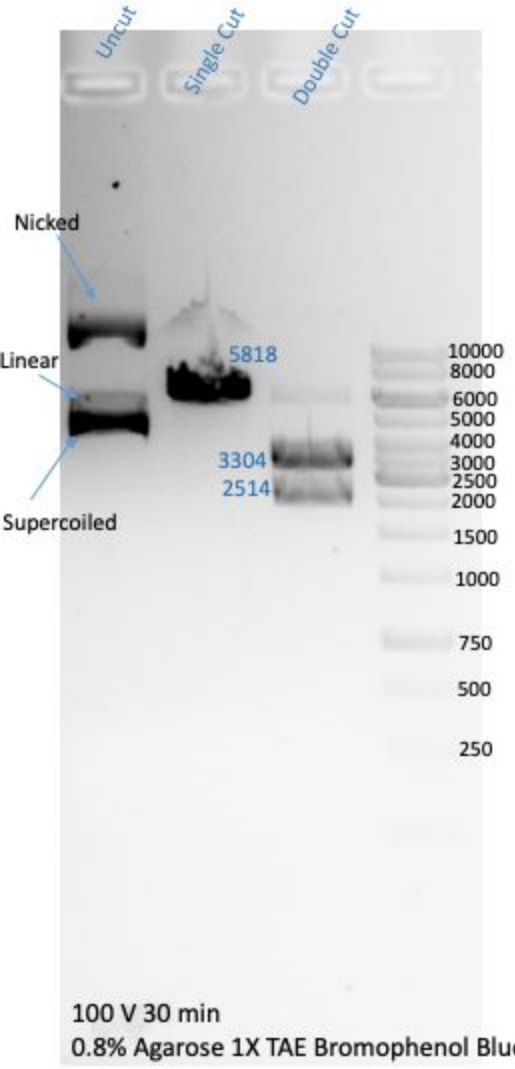


Mini-Prep



100 V 30 min 1Kb Ladder
0.8% Agarose 1X TAE Bromophenol Blue

Transformed Cells → Suspension Buffer → Lysis Buffer → Neutralization Buffer → Isopropanol Precipitation → Ethanol Wash



100 V 30 min
0.8% Agarose 1X TAE Bromophenol Blue

Restriction Digestion

Aim: To confirm the plasmid is pBABE-puro-mtwist

Principle: Using endonucleases that cut the plasmid of interest at specific places, we can confirm the isolation

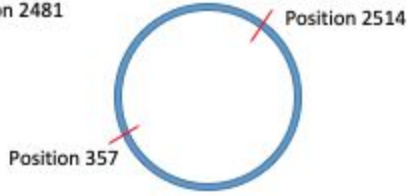
Uncut

Single Cut

BamH1

Band Length: 5818

Double Cut



XBa1

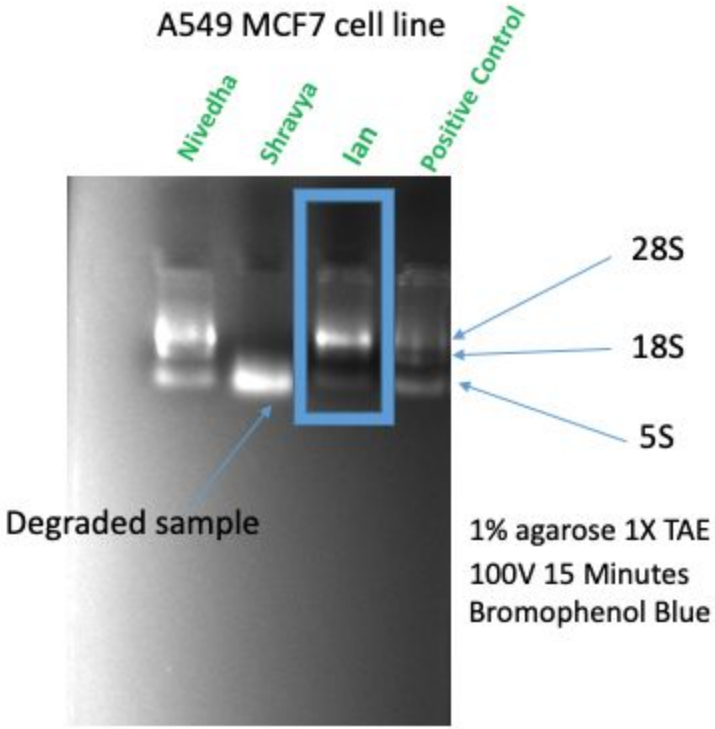
1st Band Length: 3304

2nd Band Length: 2514

RNA isolation/PCR: Overall Goal

To ultimately study the relative levels of gene expression

RNA isolation



Aim:
To learn and practice RNA isolation via TRIzol extraction method

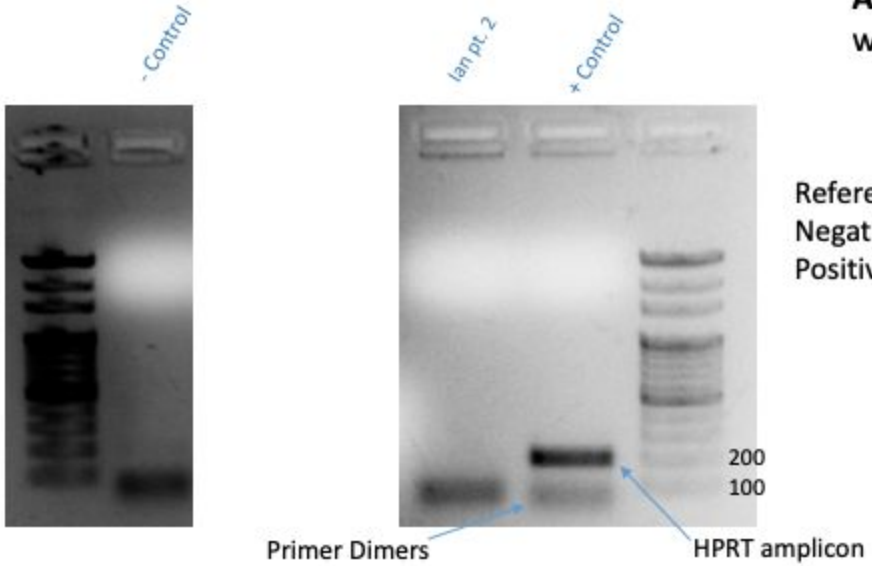
Observations:
From my sample I see a bright 28S band from large ribosomal subunit, and a faint 18S and 5S band from the small subunit.

Results:
Based on the presence of intact rRNA, my isolation was successful in not degrading the RNA.

Sample	Concentration (ng/µL)	A260/280	A260/230	Total Yield (ng in 20 µL)
A549 MCF7	184.9	1.87	2.36	3698

Harvest Cells → TRIzol → Chloroform → Isopropanol Precipitation → Ethanol Wash → Dissolve in σ water and quantify

DNase Treatment and –RT PCR



Aim: Remove DNA contaminants through DNase and confirm with PCR.

Samples: Isolated RNA from PBMC Breast cancer patient samples

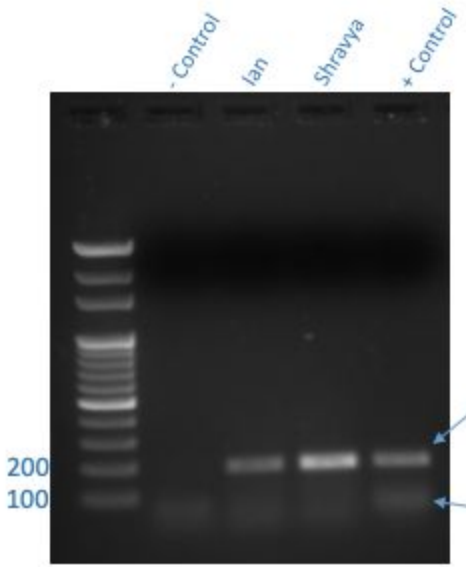
Reference gene: HPRT
Negative Control: Master mix + σ water
Positive Control: cDNA from MDA-MD 231 A76 Treated Attached cells

1.5% agarose 1X TAE xylene cyanol
100 V 30 min 100 BP ladder

*Repeated the gel due to ladder contamination

Isolate RNA → Treat 2000 ng of RNA with DNase → Run PCR for confirmation

cDNA Synthesis and +RT PCR



1.5% agarose 1X TAE xylene cyanol
100 V 45 min 100 BP ladder

Aim: To synthesize completementary DNA from isolated RNA sample

Principle: Using Reverse Transcriptase and random hexamer primers, the RNA Previously isolated can be converted into complementary dsDNA.

Reference gene: HPRT
Negative Control: Master mix + σ water
Positive Control: cDNA from MDA-MD 231 A76 Treated Attached cells

HPRT Amplicon

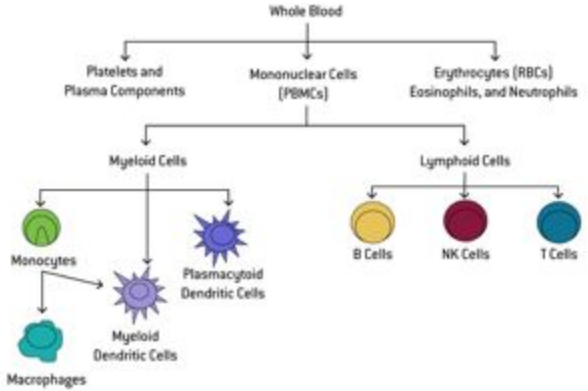
Primer Dimers

Kidwai Institute of Oncology

Bangalore, Karnataka India

Patient Blood Samples from Kidwai → Purification of PBMCs

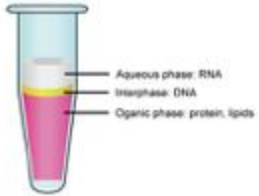
Peripheral Blood Mononuclear Cells (PBMC)

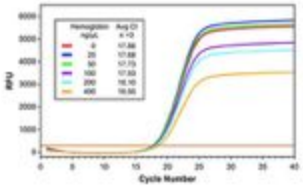


Healthy patients and cancerous patient samples

Workflow for Patient Samples

Purification of PBMCs → RNA isolation → DNase Treatment → -RT PCR → cDNA Synthesis → +RT PCR → Quantitative PCR





My Lab

IISc Campus

Travels

Thanks for listening!