University of Dundee

From GWAS to Functional Studies in South Indian Population

By

Yogaprabhu

Research Fellow

MDRF

Mentor

Dr. Radha Venkatesan

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How do I leverage our GWAS results, a goldmine of wealth ? ???

An experimental approach…

“Low levels of HDL Cholesterol is a key Metabolic difference in Indian population compared to European population”

Reinforced from WP2 data

CETP*(**Cholesteryl Ester Transfer Protein) **, *LIPC (Hepatic lipase) and ***LPL*** (Lipoprotein lipase) **WP2 RESULTS**

Analyze and find the rare variants from T2D in Indian population

Validate by Functional studies and finally prove the variants as a Functional variants or otherwise.

FUNCTIONAL CHARACTERIZATION OF GENETIC VARIANTS
(Both for EOT2D variants and HDL pathway project)

WORKFLOW

Choosing the rare variants

Creation of variants using Site Directed Mutagenesis (SDM)

Transformation (using top10 *E.coli* cells)

Confirmation of mutagenesis by Colony PCR and Sequencing

Isolation of Plasmids

Confirmation of plasmids using sequencing

Functional Studies based on the nature of gene product

GWAS TO FUNCTIONAL STUDIES

To characterize genes coding for Transcription Factors:

Reporter assay	To access the transcription activity.
Western blot	To access the protein expressions.
EMSA	To access the DNA binding activity.
Subcellular localization	To access the localization of protein.

To characterize genes coding for channel proteins:

Western blot	To access the protein expressions.
Surface expression	To check the trafficking of channel.
Thallium flux assay	To check the channel response to known inhibitors/pharmacological agents.

To characterize genes coding for enzymes:

Western blot	To access the protein expressions.
Kinetic assay	To access/measure the half life of an enzyme.
Activity assay	To access/measure the enzyme activity.

Molecular cloning of Genetic variants-SITE DIRECTED MUTAGENESIS (SDM)

Primer designing (https://www.agilent.com/store/primerDesignProgram.jsp)

1. It is recommended that you clear this form prior to loading each new sequence:

Clear Input

2. Select QuikChange® mutagenesis kit that you are using:

QuikChange® II

Help in choosing a mutagenesis kit

3. Find your DNA sequence by pressing

Load a file from your hard-drive:

No file selected | Browse_

Or, paste plain text or FASTA-formatted DNA sequence in the box below:

4. Load it.

Upload Now - or - Upload Translated

or specify a DNA region to translate from ___ to ___ Upload Translated Region

Finally, obtain your designed primer sequences.

Design Primers

Site Directed Mutagenesis using *Quick-change II Site-Directed Mutagenesis Kit*

Transformation (*E.coli* top10 competent cells)

Colony PCR

Cycle sequencing

Confirmation of variants

Plasmids isolation

Plasmids will be ready

TRANSACTIVATION ASSAY

Cells will be plated

Incubate at CO_2 incubator at 37˚C for 18-20hrs

Confluency (70%-80%)

Cells will be transfected

Cells will be Incubate at CO_2 incubator at 37˚C for 20-24hrs

Cell Lysate will be prepared by using Passive Lysis Buffer(PLB)

Reporter Assay

Wild type-pcDNA 3.1-WT

Target DNA -pGL3-target DNA

Internal control-pRL -SV40

Variant- pcDNA3.1-V1

WESTERN BLOTTING

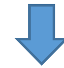
Cells will be plated

Confluency (70%-80%)

Cells will be transfected (Wild type & variant)

Incubate at CO_2 incubator at 37˚C for 24-36hrs

Cell Lysate will be prepared by using RIPA Lysis Buffer in cold conditions

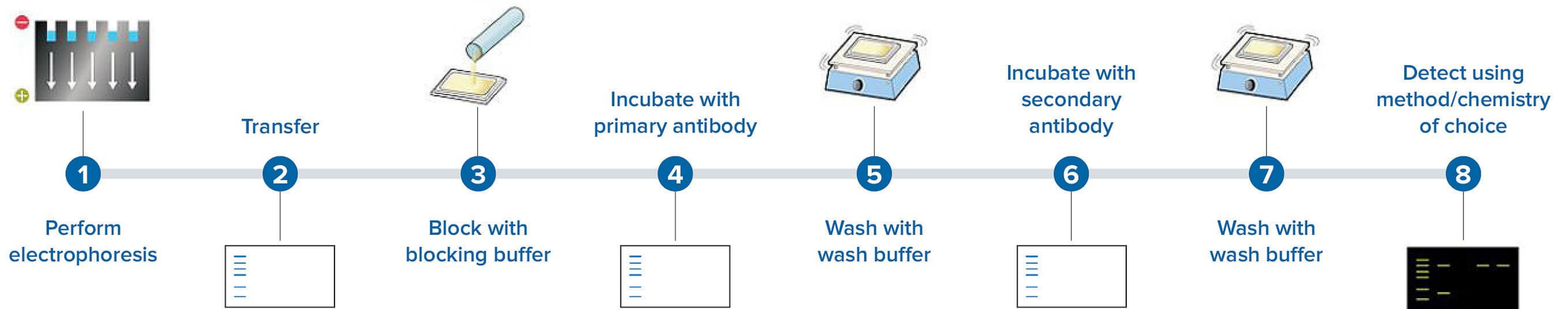


SDS-PAGE

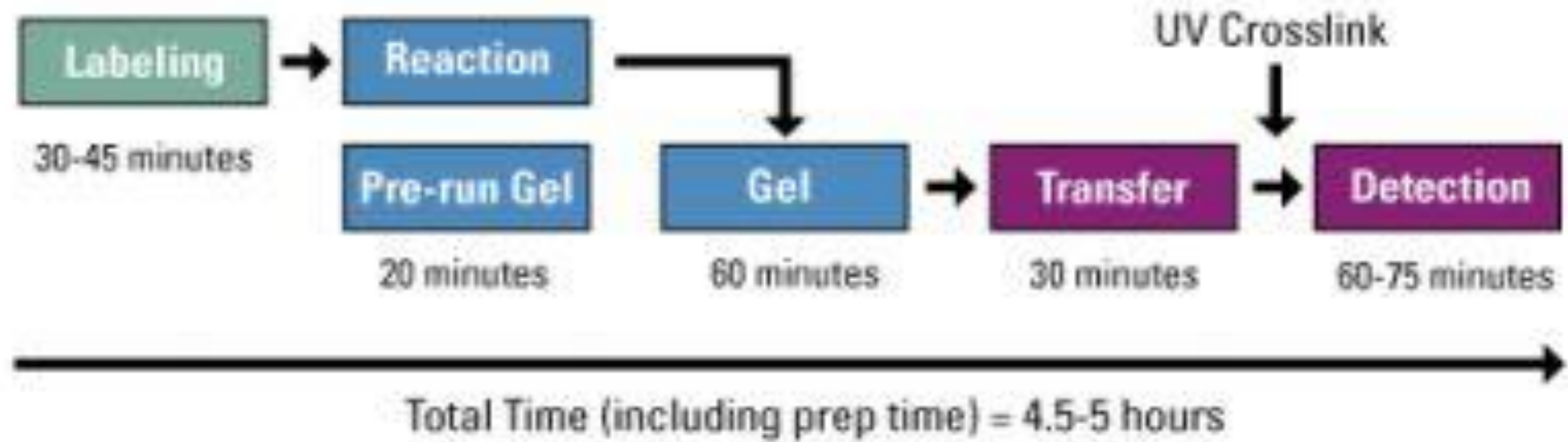
Ctnd……

WESTERN BLOTTING OR IMMUNOBLOTTING

- Tissue preparation — RIPA Lysis buffer
- Gel electrophoresis — SDS-PAGE (Polyacrylamide Gel Electrophoresis)
- Transfer — To the membrane (PVDF orNitrocellulose membranes)
- Blocking — Bovine serum albumin
- Detection — Primary and secondary antibody
- Analysis



Electrophoretic Mobility Shift Assay (EMSA)



Electrophoretic Mobility Shift Assay: Analyzing Protein - Nucleic Acid Interactions
Carolina Alves and Celso Cunha

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