One Minute Pitch

The **high sensitivity** of RNAscope Technology coupled with its ability to reliability detect **ANY gene *in situ*** , makes it ideal to **identify and localize** stem cell populations, **characterize** stem cell markers (maintenance or regeneration) and **identify** the signals secreted from stem cells.

Typical challenges with stem cell markers and sweet spots for RNAscope (a) newly discovered & require validation, (b) lack antibodies and/or (c) expressed at low levels in rare stem cell populations.

Pain points & Our Benefits as a Solution

Elucidate populations	**Context** Identify, characterize, & localize stem cell populations
Low expressing or rare	**Sensitivity** Detect low expressing genes & identify rare stem cell populations
Interactions	**Multiplex** Enables scientists to look at markers for stem cell maitenance & those of regeneration
No antibodies	**Any Gene** Many stem cell markers do not have antibodies
Gene family	**Any Gene** Enables study of multiple genes in a family
Secreted Proteins	**Any Gene** Reliably detect secreted proteins and its target receptors.
Validate	***In situ*** expression enables newly discovered markers to be validated within morphological context
Noncoding RNA	**LncRNA** capable addresses expression of this RNA species

Key Tools

URL acdbio.com/stemcell
Spotlight Interview Anne Marie Baker(Showpad)
Presentation Stem Cell Slide Deck (Showpad)
2 Application Notes in Showpad
1. Cellular localization of RNA Expression in Stem Cells using RNAscope® Technology

Key Tools (cont)

2. Visualization of Lgr5+ stem cells and the immune response in the inflamed mouse colon by the RNAscope® in situ hybridization assay
Dual ISH + IHC Turkekul *et al* Methods Mol. Biology, 2017
LncRNA Boo *et al. Nature Comm, 2015 linc1253
Single-Cell RNAseq Validation Joost *et al* Cell Systems, 2016

KOL Reference (Name to Drop)

Roel Nusse Principal Investigator at Stanford University
KOL in WNT signalling, Stem Cell and Developmental Biology
His lab's work is seminal in the discovery of WNT signaling.
2012 publication Science used RNAscope to visualize over 15 WNT family genes. RNAscope continues to be one of their mainstream applications, Nusse is senior author in ~10 RNAscope publications.
See Pubcrush for the following:
Lim *et al.* Science, 2012 (15+ Wnt genes, Axin, Dkk in epidermal tissues)
Tan *et. al* PNAS 2014 (19 Wnt genes in bone tissues)
Lim *et al* PNA 2016, (Wnt family in hair follicles)

Gene Target LGR5--No Antibodies

LGR5 is a member of the Wnt signaling pathway, often investigated with R-spondin & Wnt-3a, LRP6 and FZD5

LGR5 shown to be a tumor suppressor gene, and that its main role is delimiting stem cell expansion in their respective niches

LGR5 expression levels observed to indicate different stages of gastrointestinal cancers, which suggests that the histoanatomical distribution of LGR5+ve stem cells determine how the cancer advances. So understand morpholigical context with expression is important.

Over 30 LGR5 RNAscope papers published, LGR5 publication list in Showpad

Spotlight interview of Anne Marie Baker, Cancer Research UK discussing her publication in Nature using RNAscope

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Neural Stem Cell Markers

Neural stem cells (NSCs)	Three Major Cell Signaling Pathways: Wnt/β-catenin pathway, Notch signaling, Shh-Gli signaling
NSC Self-Renewal	HES1/5, CBF-1,SOX2, HMGA2, BMI-1,Gli-2/3,TLX
NSC Growth	SoxB1, GLI family, HES1 and HES5, BMI-1,HESR1 and HESR2, REST
NSC Differentiation	Long list...SOX4-11, SOX17, MASH1, NGN3,PITX3, FOXA1/A, NGN2

Background: Concepts & Vocabulary

Stem cell	Undifferentiated biological cells that can differentiate (Potency) into specialized cells and can divide (through mitosis) to produce more stem cells (Self-renewal).
Self-renewal	Ability to go through numerous cycles of cell division while maintaining the undifferentiated state
Potency	Capacity to differentiate into specialized cell types. totipotent or pluripotent—to be able to give rise to any mature cell type
Organoids	an artificially grown mass of cells or tissue that resembles an organ. Used as tools to study organ development.
Lgr5-eGFP mice	Use these mice as an alternative to identify LGR5+ cells. These researchers are strong targets as they probably don't know that RNAscope can directly detect LGR.

Potency is also described as the gene activation potential within a cell which like a continuum begins with totipotency to designate a cell with the most differentiation potential, pluripotency, multipotency, oligopotency and finally unipotency

Type 1 Embryonic Stem Cell

Embryonic stem (ES) cells are the cells of the inner cell mass of a blastocyst (4–5 days post fertilization), an early-stage embryo.
ES cells are pluripotent (has potential to become any of the 200 cell types) and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm.
Research applications-- uses mouse and human embyonic stem cells (fertilized *in vitro*) to
(1) **determine the presence of transcription factors** such as Nanog and Oct4 that turn genes on and off at the right time, important in cell differentiation and embryonic development. Both are associated with stem cell maintainance and self-renewal.
(2) **determine the presence of particular cell surface markers** that are typically produced by undifferentiated cells.

Type 2 Non-embryonic/ Adult/ Somatic Stem Cells

Adult stem cells also called somatic stem cells maintain and repair the tissue in which they are found in children and adults
Pluripotent adult stem cells are rare
Research of adult stem cell is richer, less controversial and aimed to characterize their potency and self-renewal capabilities.
Adult stem cells include:
Hematopoietic stem cells give rise to all the types of blood cells
Mesenchymal stem cells present in many tissues. Those from bone marrow (bone marrow stromal stem cells, skeletal stem cells) give rise to a variety of cell types: bone cells (osteoblasts and osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and stromal cells that support blood formation.
Neural stem cells give rise to neurons, astrocytes and oligodendrocytes.
Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, Paneth cells, and enteroendocrine cells.
Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles.

Type 3 Induced Pluripotent Stem Cells (iPSCs)

Induced pluripotent stem cells, iPSCs reprogram adult stem cells to become like embryonic stem cells. They can differentiate into all types of specialized cells in the body. This means they can potentially produce new cells for any organ or tissue. To create iPSCs, scientists genetically reprogram the adult stem cells so they behave like embryonic stem cells.
Researchers would need to validate targets typical of embryonic stem cells

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Type 4 Cord Blood Stem Cells

Cord blood stem cells and amniotic fluid stem cells

Cord blood stem cells are harvested from the umbilical cord after childbirth. They can be frozen in cell banks for use in the future. These cells have been successfully used to treat children with blood cancers, such as leukemia, and certain genetic blood disorders.

Probing Questions for Adult Stem Cell Researchers

How many different kinds of stem cells are you interested in and in which tissue do they exist?

Are these adult stem markers "leftover" embryonic stem cells, or do they arise in some other way? Are they common or rare populations?

Are you examining stem cell maintenance—which markers? Antibodies? LncRNA? Secreted Proteins? Low expressing/rare?

Are you interested in stem cell renewal—which markers? Antibodies? LncRNA? Secreted Proteins? Low expressing/rare?

Are you examining mechanisms driving differentiation? What genes are activated to signal differentiation? Is differentiation influenced by the state of the neighboring cells? Are any of these secreted proteins. Is the location of the receptors of the secreted proteins important?

Are you looking at factors/genes that stimulate stem cells to relocate to sites of injury or damage?

What is known about the expression level of your gene target in your tissue of interest?

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