GenScript
Make Research Easy

Rabbit Monoclonal Antibodies (mAbs) in Tissue Diagnostics

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Outline

Outline

Introduction to Rabbit mAb

Generation of Rabbit mAbs

IVD - Tissue Diagnostics

Rabbit mAbs in Tissue Based IVD

GenScript Antibody Services

Common Issues and Concerns with Rodent mAbs

Immunoreactivity

Is your antigen not eliciting an immune response in mice?

Sensitivity

Do you have low affinity against difficult antigens?

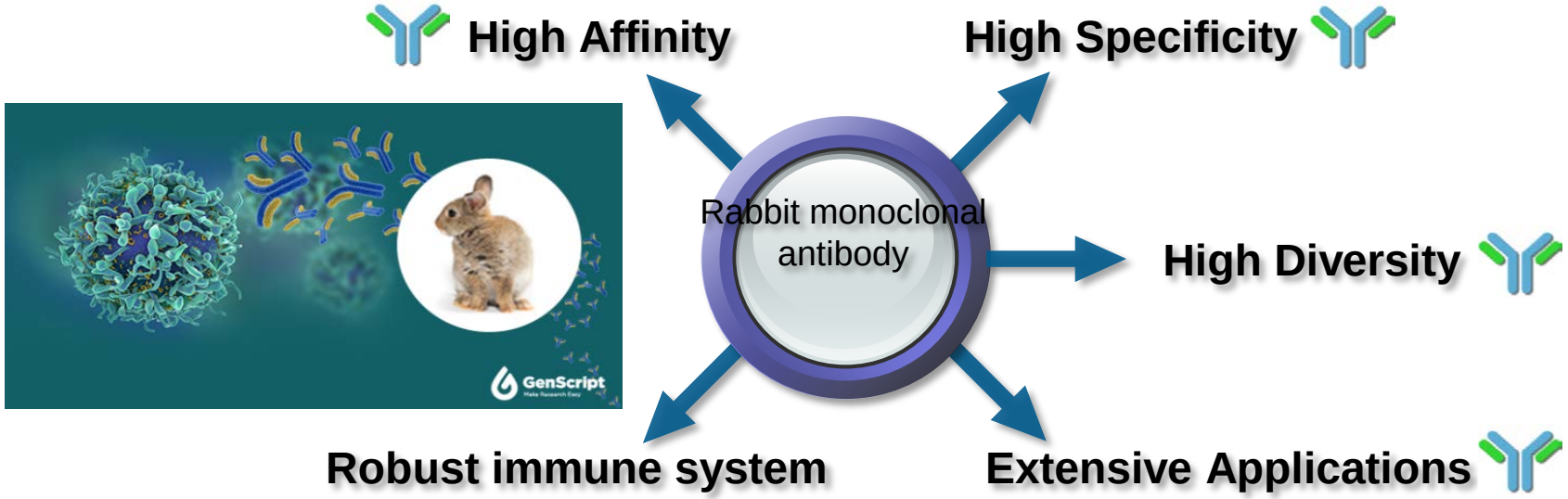
Specificity

Do you have difficulty discerning between similar epitopes?

Rabbits Can Provide Solutions

- Kd typically at the picomolar (10^{-12}) level [mouse mAbs: Kd at nanomolar (10^{-9}) level]
- Permit higher working dilutions (5 -10 times)

- Able to distinguish between very similar proteins or sequences
- Lower cross reaction



- Recognizes wider range of epitopes, novel epitopes
- More immuno-responsive to small epitopes (<1kDa)
- Bigger spleen
- Effective against antigens that are not immunogenic in mice

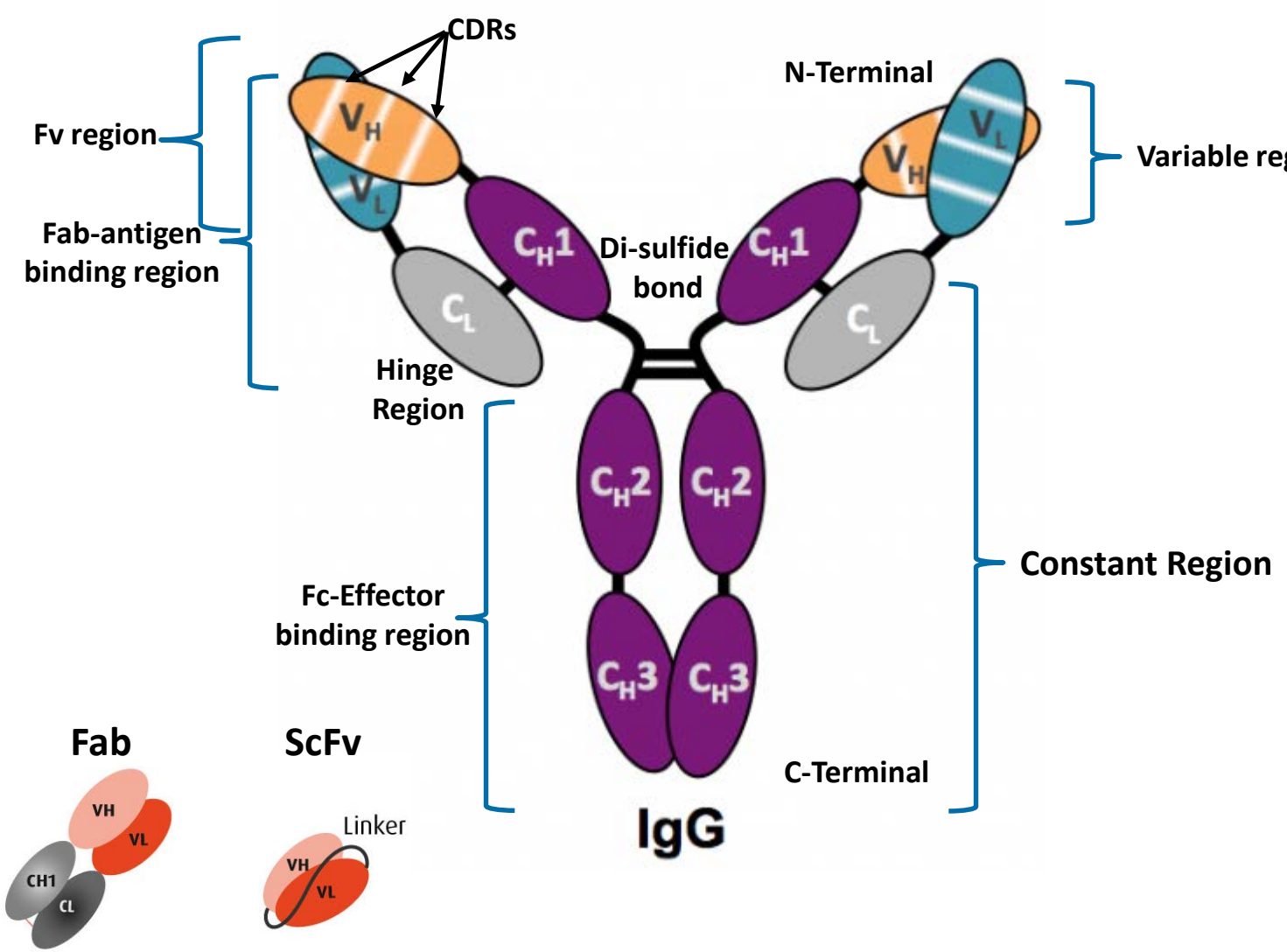
- ELISA, Westerns, FACS, IP, ICC…
- Excellent results in IHC
- Good for IVD/anti-idiotype Ab…

Rabbits for Antibody Generation

- The evolutionary distance of rabbits from human is further than that of rodents, allowing the rabbit immune system to generate mAbs against human antigens commonly non-immunogenic in mice
- High genetic heterogeneity compared to inbred strains of mice
  - New Zealand white strains: research activities
  - European Rabbits: b9 κ-light-chain, a very rare allotype, source for therapeutic mAbs
- Different body size and mechanisms to generate primary and secondary Ab repertoires
- Presence of CD1 Ag-presenting molecule and expression of all 5 CD1s leads to a strong immune response against small molecules, whereas mice/rats only express CD1d
- Presence of sequence diversification in Rabbit Ab repertoire

Group 1 CD1 Genes in Rabbit; Sandra M. Hayes and Katherine L. Knight; Journal of Immunology 2001

Structure and Function of Immunoglobulins

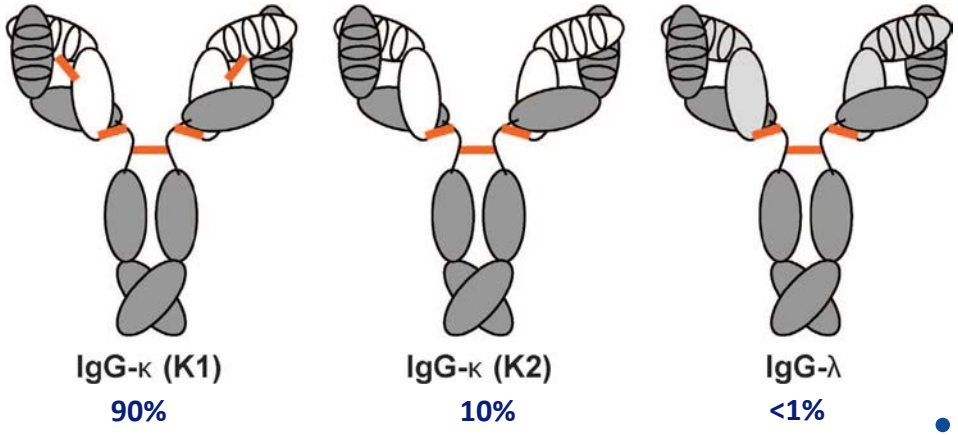


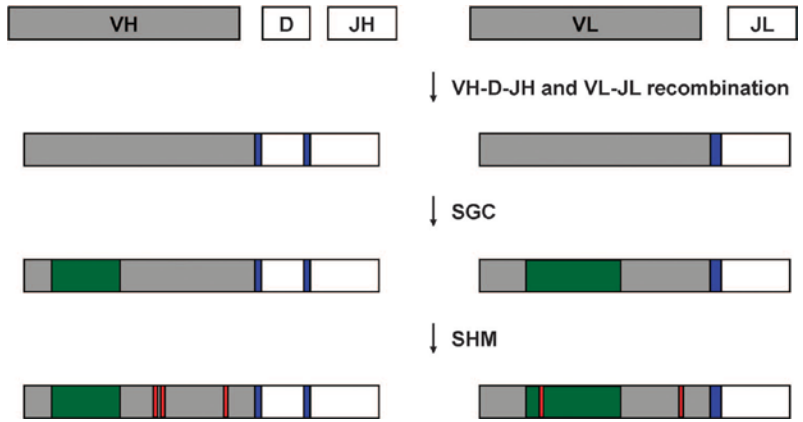
- Glycoproteins
- Comprised of two regions
- Variable regions binds to specific antigens
- Constant regions determine structure and immune function

CDRs : Complementarity determining regions
CH : Constant domain, Heavy chain
CL : Constant domain, Light chain
VH : Variable domain, Heavy chain
VL : Variable domain, Light chain

Schematic of Rabbit Antibodies in IgG Format & Molecular Sequence Diversification

Unique IgG structure





Weber et al., Experimental & Molecular Medicine (2017) 49

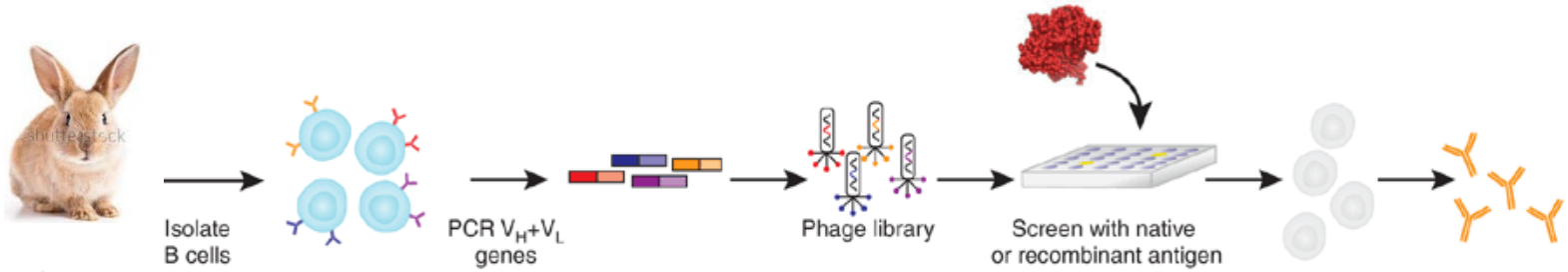
- Highly diversified primary and secondary B cell repertoire
- Special intra-chain disulfide bond in light chain: increased stability

- CDRs have more variations than rodent and human
  - Longer HCDR3 : rabbit 15+ 4aa, mouse 11+ 2aa, human 15+ 4aa
  - Longer LCDR3: rabbit 11 + 2aa, human and mouse 09 + 1aa
- More diversified LC: κ (K1 & K2) and λ (4 K1 allotypes b4, b5, b6 & b9)
  - Blue: N-nucleotides addition:(non-templated nucleotide)
  - Green: SGC (somatic conversion): Rabbits 23% others (0.1 – 2.5%)
  - Red Color: SHM (Somatic hyper mutation)

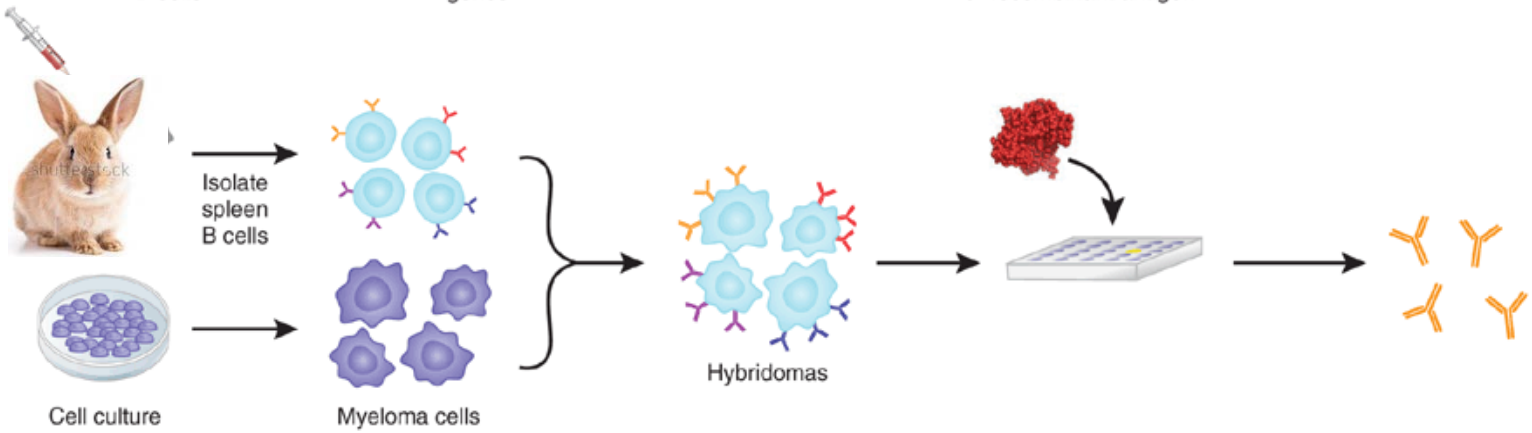
Outline

Technologies for Generating Rabbit mAbs

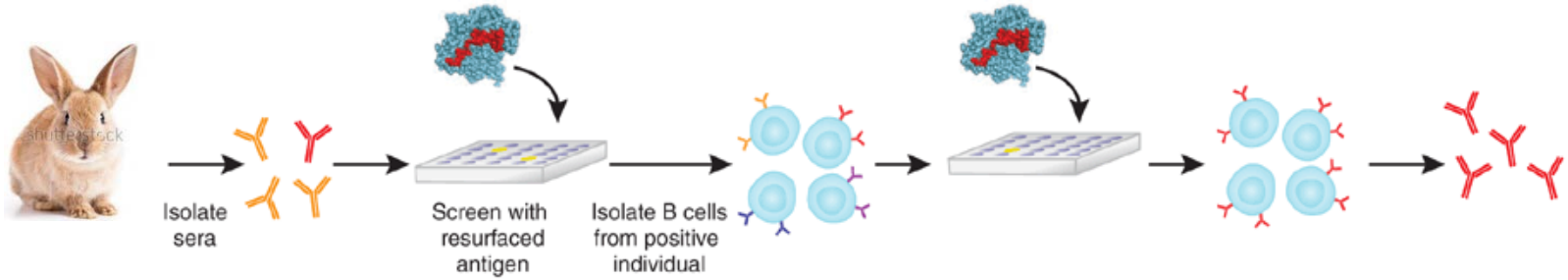
Phage Display



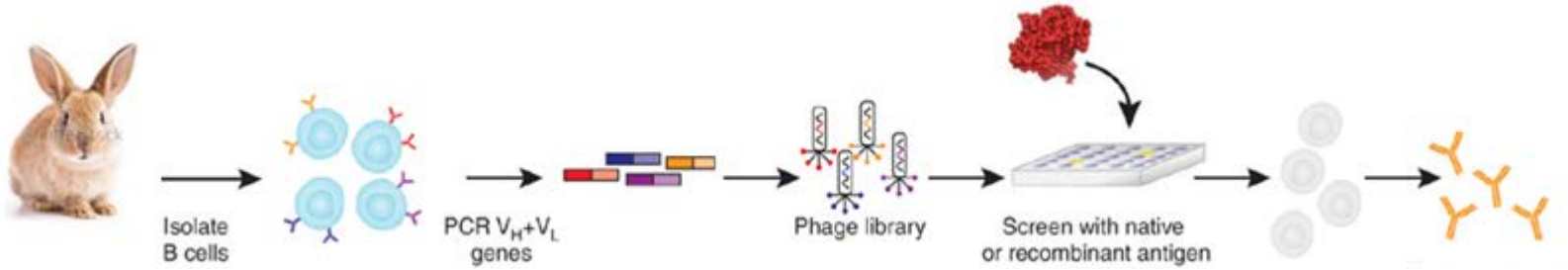
Hybridoma



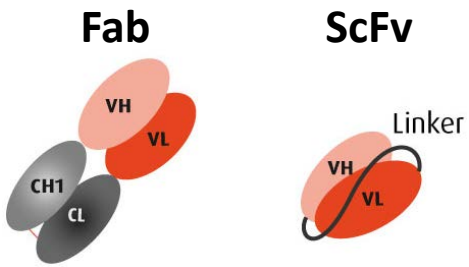
Single B-cell Cloning



Phage Display



- Phage display library: V gene harvested from Lymphocytes (from RNA to cDNA)
- Combinations of VHs and VLs cloned - ScFV (cDNA to construct library)
- Expressed on the surface of bacteriophage coat protein by fusion
- Specific mAbs expressing phage particles on their tips are selected and used
- Loss of natural cognate pairing of VHs and VLs



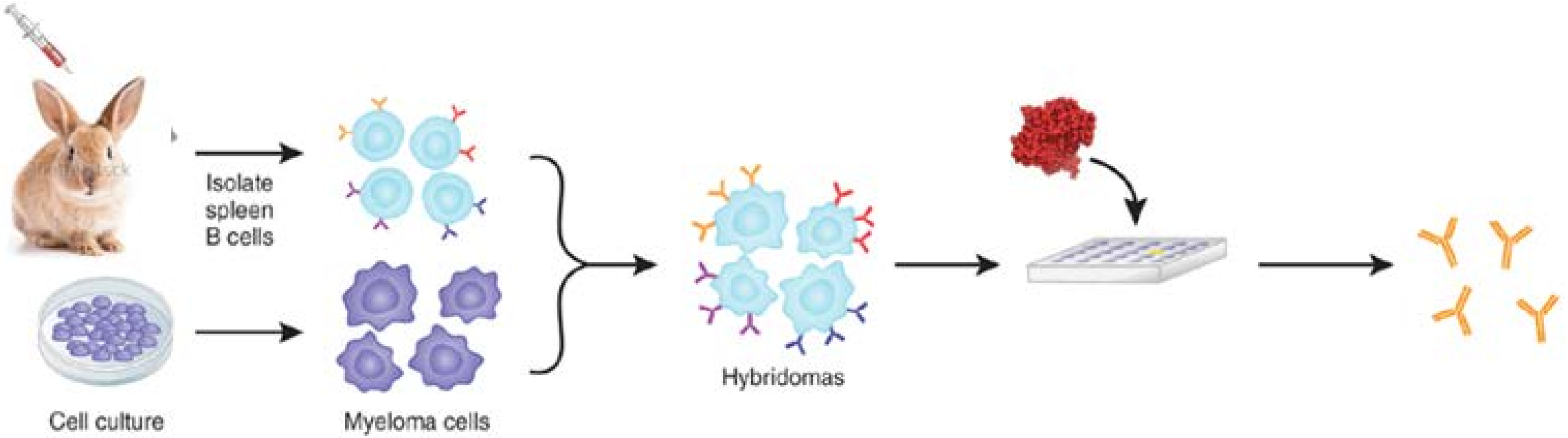
Advantages

- Faster steps with variable regions when compared to hybridoma – 6 weeks
- Generates multiple binders for different antigens
- Further genetic engineering – affinity maturation and specificity optimization (Immunotherapy)

Disadvantages

- Fab is better than ScFv
- Loss of natural cognate pairing of H & L chain
- Expensive compared to hybridoma technology
- Identifies binders regardless of the immunogenic property

Hybridoma Technology



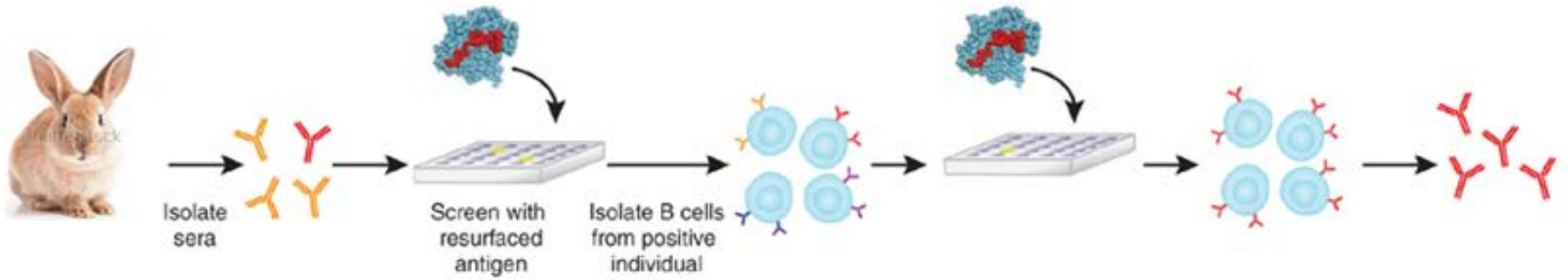
Advantages

- Excellent immunogenic property
- Less expensive among other technologies
- Further genetic engineering – affinity maturation and specificity optimization

Disadvantages

- Duration is long – 5 months
- Low efficiency of cell fusion
- Multiple binders for one antigen

Single B-cell Cloning & Single Plasma Cell Cloning



- B-cells using FACS
- Performing single cell RT-PCR with antibody specific primers
- Amplification of IgG genes with PCR and sequencing
- Amplification of IgG genes expressed in bacteria or in CHO cells
- Antibodies purified and screened

Advantages

- Faster steps compare to phage display – 3 weeks
- Widely used in therapeutics antibodies
- Preserving the natural cognate pairing
- Generate mAbs with attractive affinity
- Further genetic engineering

Disadvantages

- Very expensive, compare to phage display

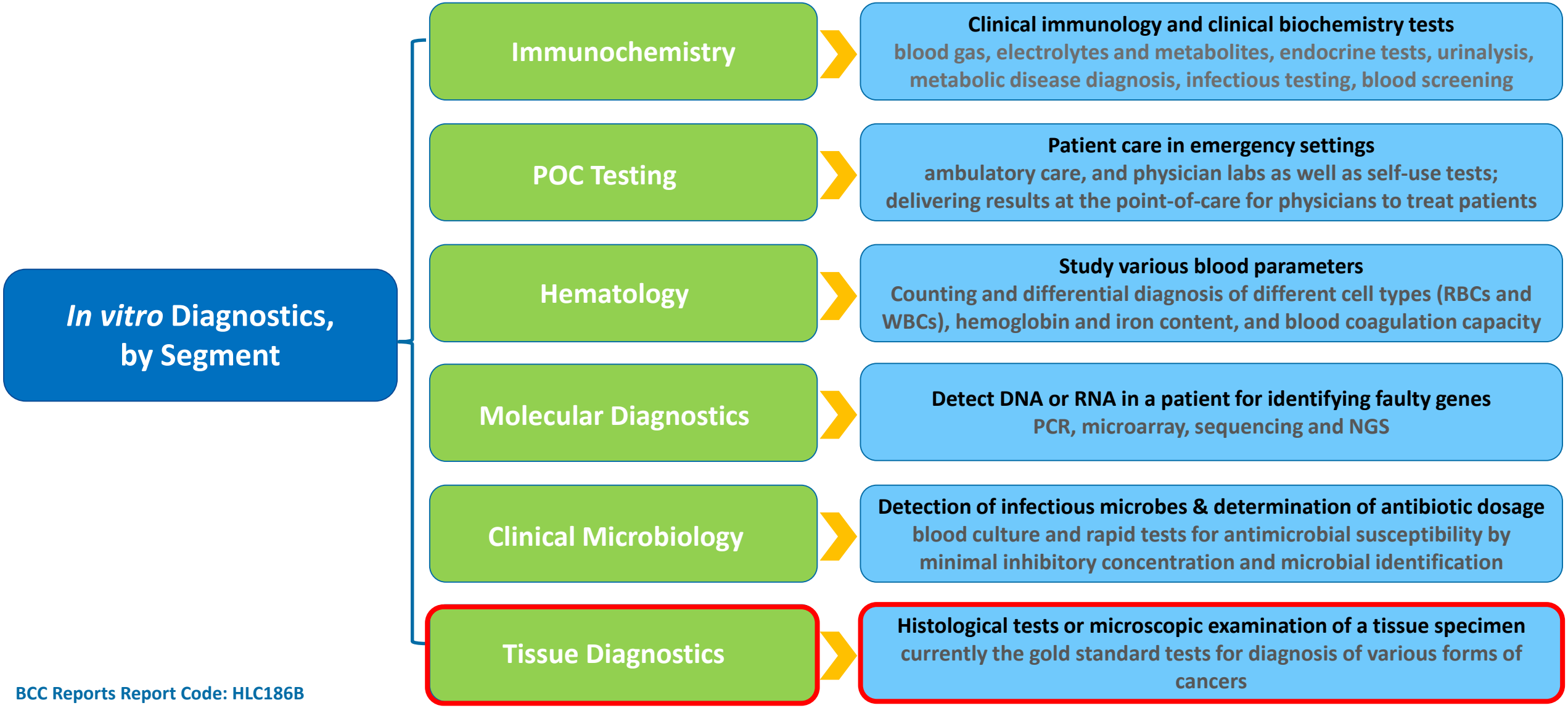
A Quick Comparison

	Phage display	Hybridoma	Single B cell
TAT	Medium	Medium	Medium-short
Chain pairing	Mixed	Naive	Naive
Affinity	Medium-low	High	High
Diversity	Medium-low	Medium-high	High
Cost	Medium-high	Low	High

Combining quality, cost & turnaround time, the hybridoma technology is the most cost-efficient option.

Outline

In Vitro Diagnostics BY SEGMENT



BCC Reports Report Code: HLC186B

Tissue Diagnostics: Segments

Diagnostic tests where a small sample of tissue is used to determine the presence or absence of cancer, infection or inflammation

User

- Hospitals
- Diagnostic Laboratories
- Research Organizations
- Contract Research Organizations (CROs)
- Biotechnology Companies

Disease

- Cervical Cancer
- Lung Cancer
- Breast Cancer
- Gastric Cancer
- Pancreatic Cancer
- Lymphoma
- Others

Application

- Cancer Diagnosis
- Tissue Typing
- Pathogen Detection
- Automation

Anatomical pathology

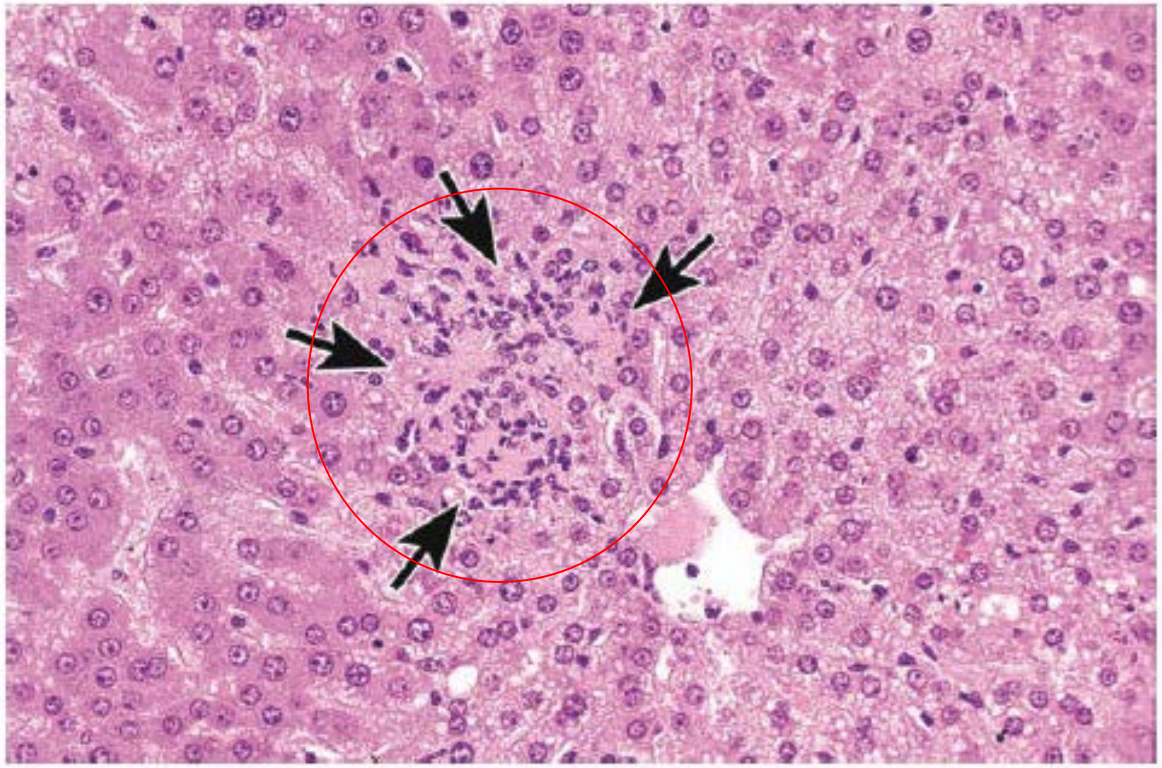
Hematoxylin and Eosin (H&E)
Immunohistochemistry (IHC)
In situ hybridization (ISH)
Digital Pathology

Anatomical pathology: a specialty that is concerned with the diagnosis of disease based on the macroscopic, microscopic, biochemical, immunologic and molecular examination of organs and tissues

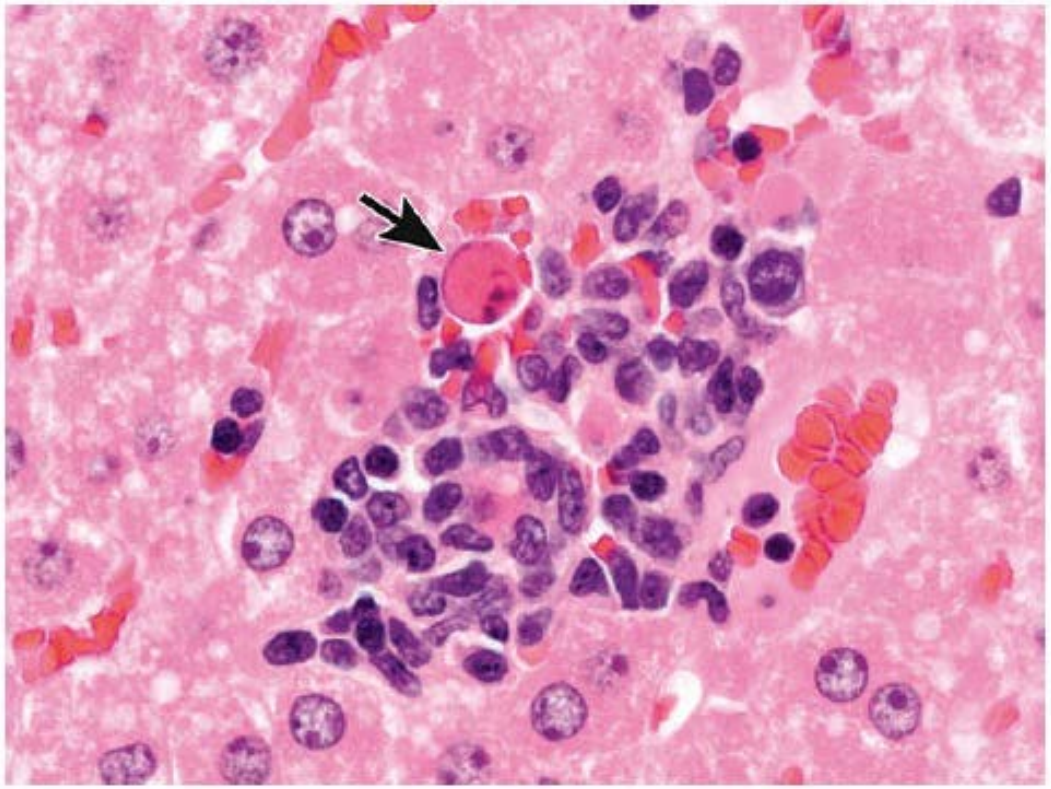
Hematoxylin & Eosin Staining

- **Hematoxylin** - deep blue/purple color, stains nucleic acids & **Eosin** – pink, stains proteins non-specifically
- In a typical tissue, nuclei stain blue whereas cytoplasm and extracellular matrix have varying degrees of pink staining

Liver tissue – focal necrosis and inflammation

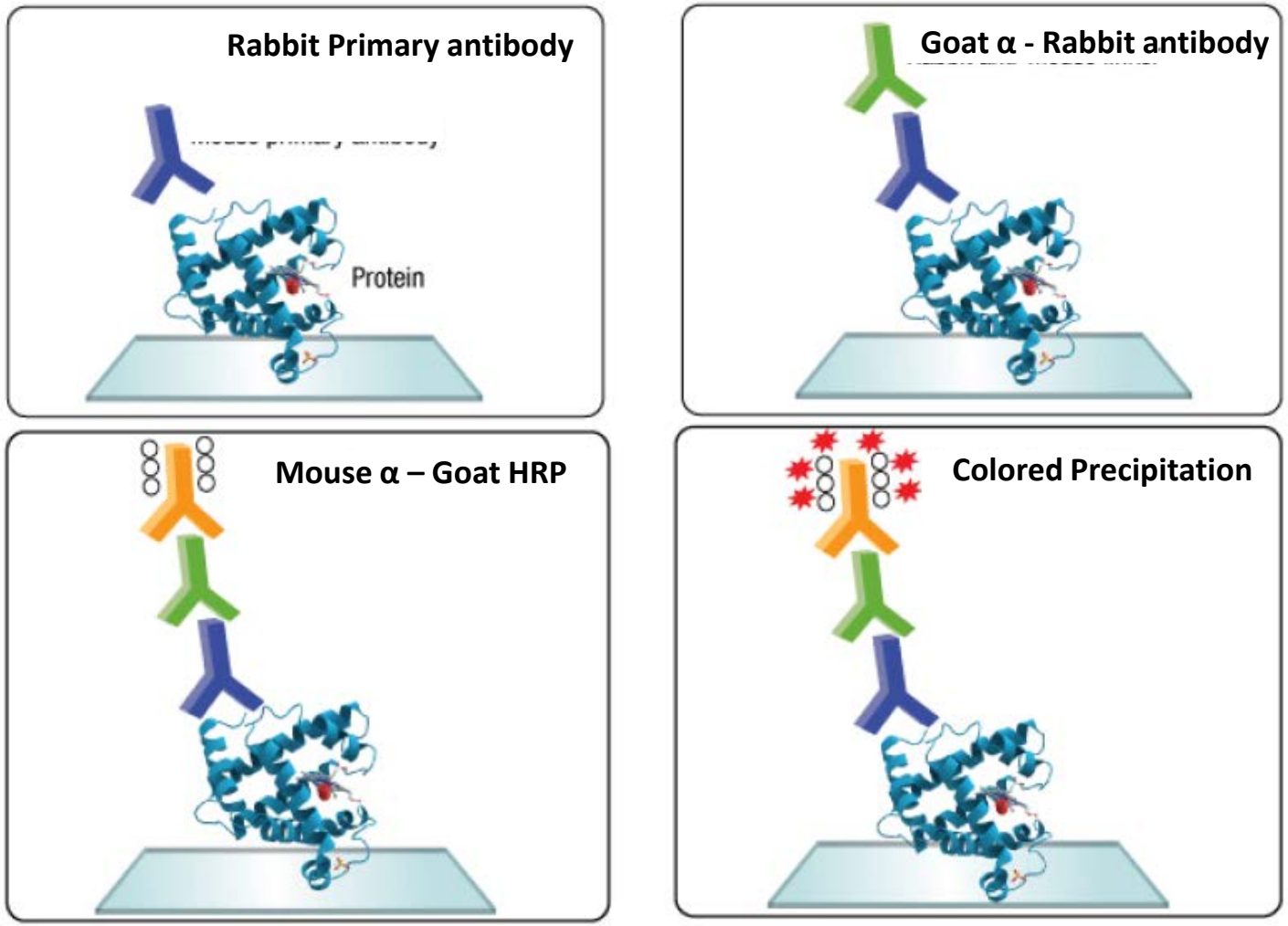


Liver tissue – apoptotic cell did not rupture

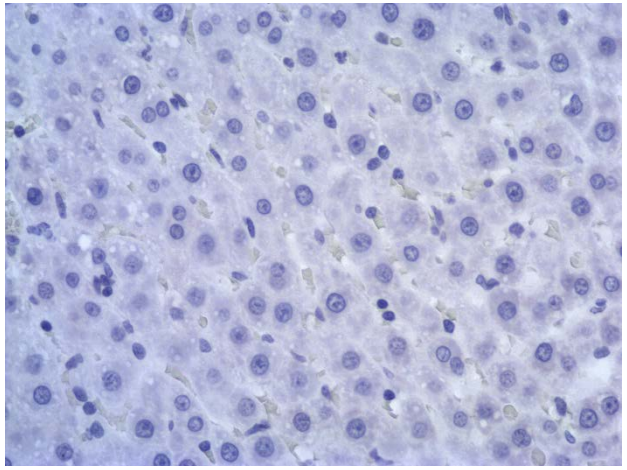


Immunohistochemistry (IHC)

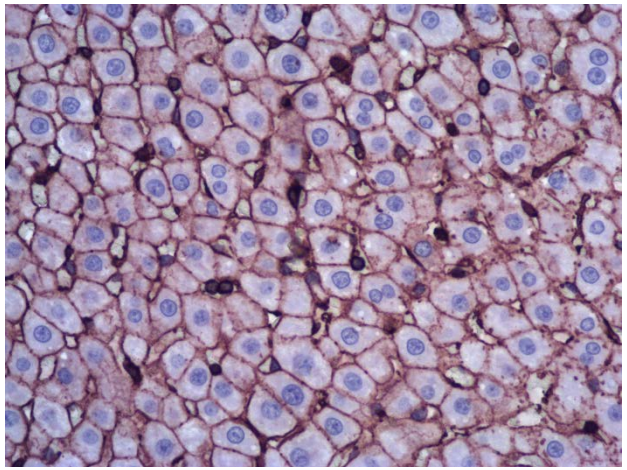
Selectively imaging antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues



Rabbit IgG Isotype Control

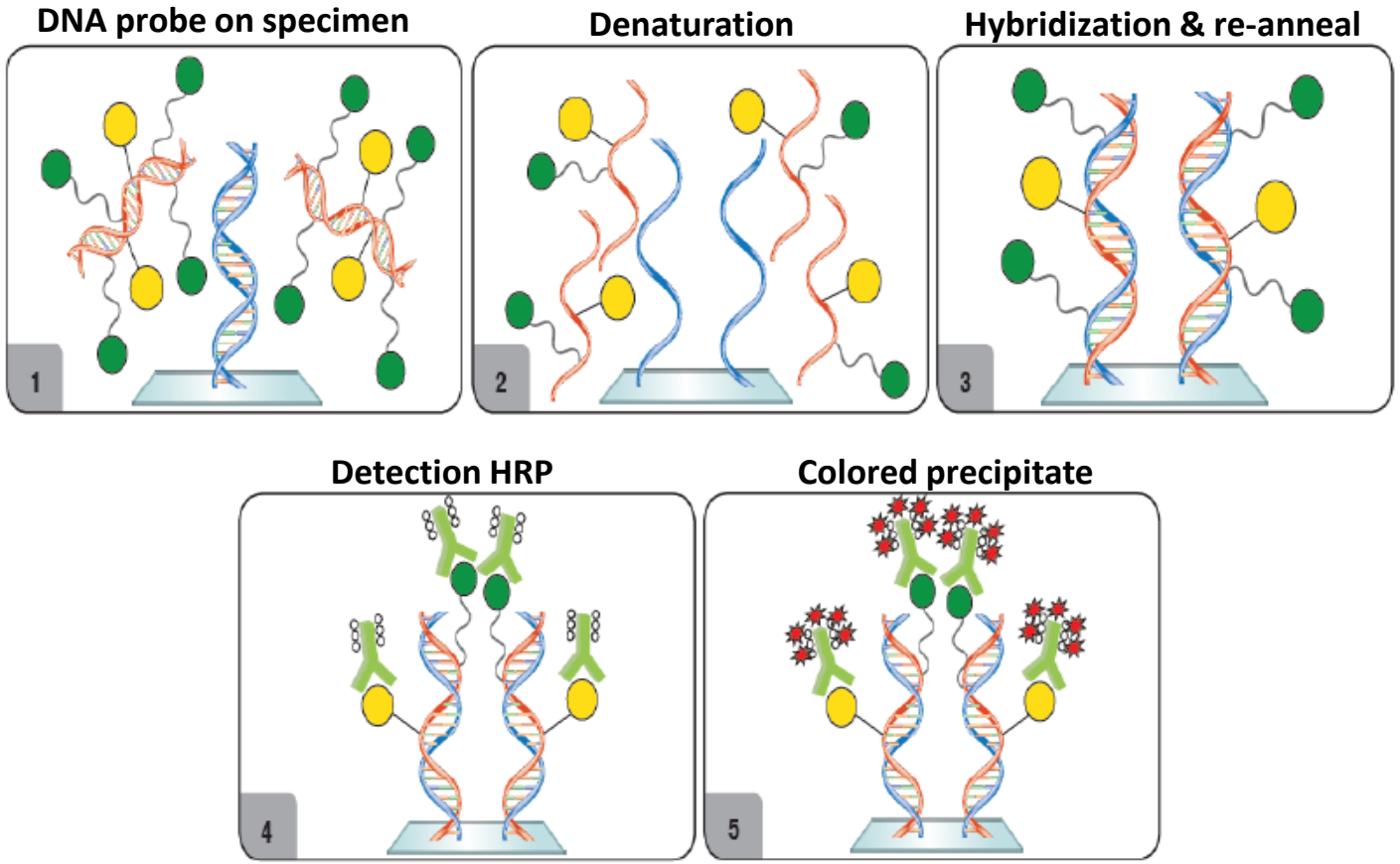


Rabbit beta-actin mAb

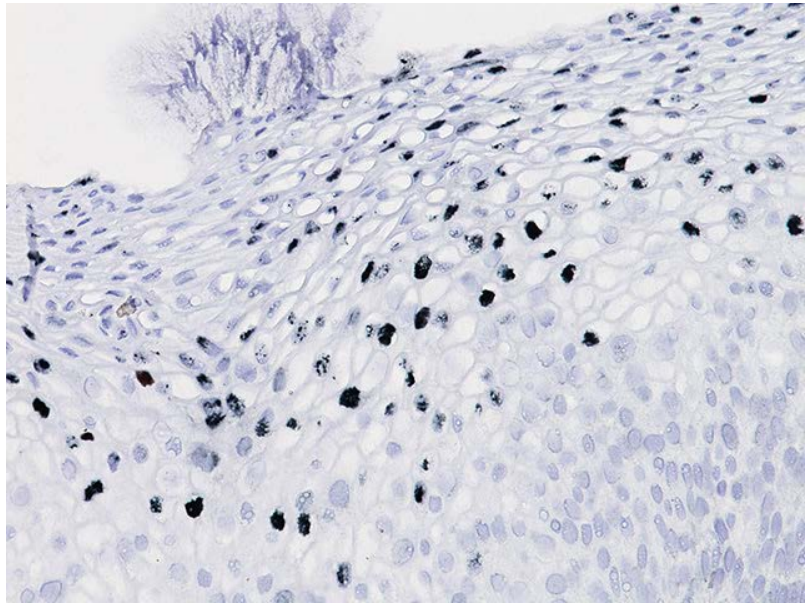


In situ Hybridization (ISH)

Reveals the location of specific nucleic acid sequences on chromosomes or in tissues, for understanding the organization, regulation, and function of genes



In situ hybridization (ISH)



Digital Pathology

Robust, reproducible, and quantitative assessment of biomarker content in the tissue context

Pathologist

ADVANTAGES

- Advanced ability to interpret architecture

CHALLENGES

- Limitations in continuous score computation
- Visual and cognitive traps and unintentional bias
- Decision varies from one to another

Algorithm

ADVANTAGES

- Computational power
- Objective and consistent staining rule sets

CHALLENGES

- Lack of cognitive complexity to robustly interpret tissue architecture

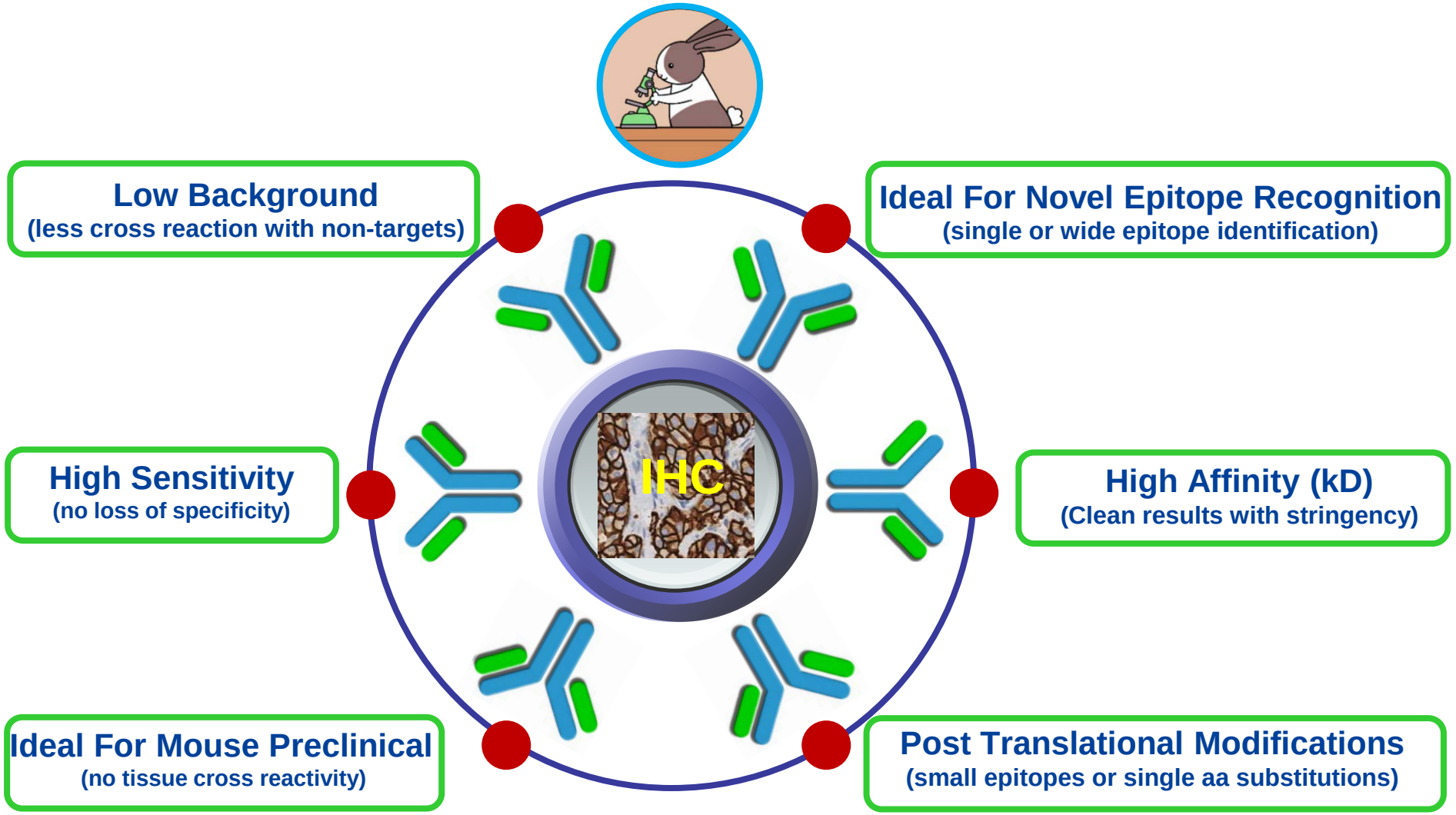
FDA-Approved Rabbit mAb-Based Diagnostic Tests

Eligible tests for treatment approval

Device Name	Diagnosis	Company	Date Approved
PD-L1 (SP263) Assay	NSCLC,non-squamous NSCLC, Urothelial Carcinoma	Ventana Medical Systems, Inc.	May 01, 2017
PD-L1 (SP142) Assay	NSCLC, UC	Ventana Medical Systems, Inc.	Oct 18, 2016
PD-L1 (SP142) CDX Assay	NSCLC, UC	Ventana Medical Systems, Inc.	May 18, 2016
PD-L1 IHC 28-8 PharmDx	NsNSCLC, SCCHN, UC & Melanoma	DAKO North America, Inc	Jan 23, 2016
Anti-Estrogen Receptor (SP1)	Breast Cancer	Ventana Medical Systems, Inc.	Dec 17, 2012
Anti-Progesterone Receptor (1E2)	Breast Cancer	Ventana Medical Systems, Inc.	Nov 16, 2011
Anti-Helicobacter Pylori (SP48)	Gastric Ulcers	Ventana Medical Systems, Inc.	Aug 25,2011
Anti human Estrogen Receptor alpha Clone SP1	Breast Cancer	DAKO North America, Inc	May 8, 2009
Anti-Human Estrogen Receptor Clone SP1	Breast Cancer	Lab Vision Corp	June 27,2006
Anti-Human Progesterone Receptor Clone SP2	Breast Cancer	Lab Vision Corp	April 24, 2006
Anti-HCR2/NCU	Breast Cancer	Ventana Medical Systems, Inc.	Nov 28, 2000

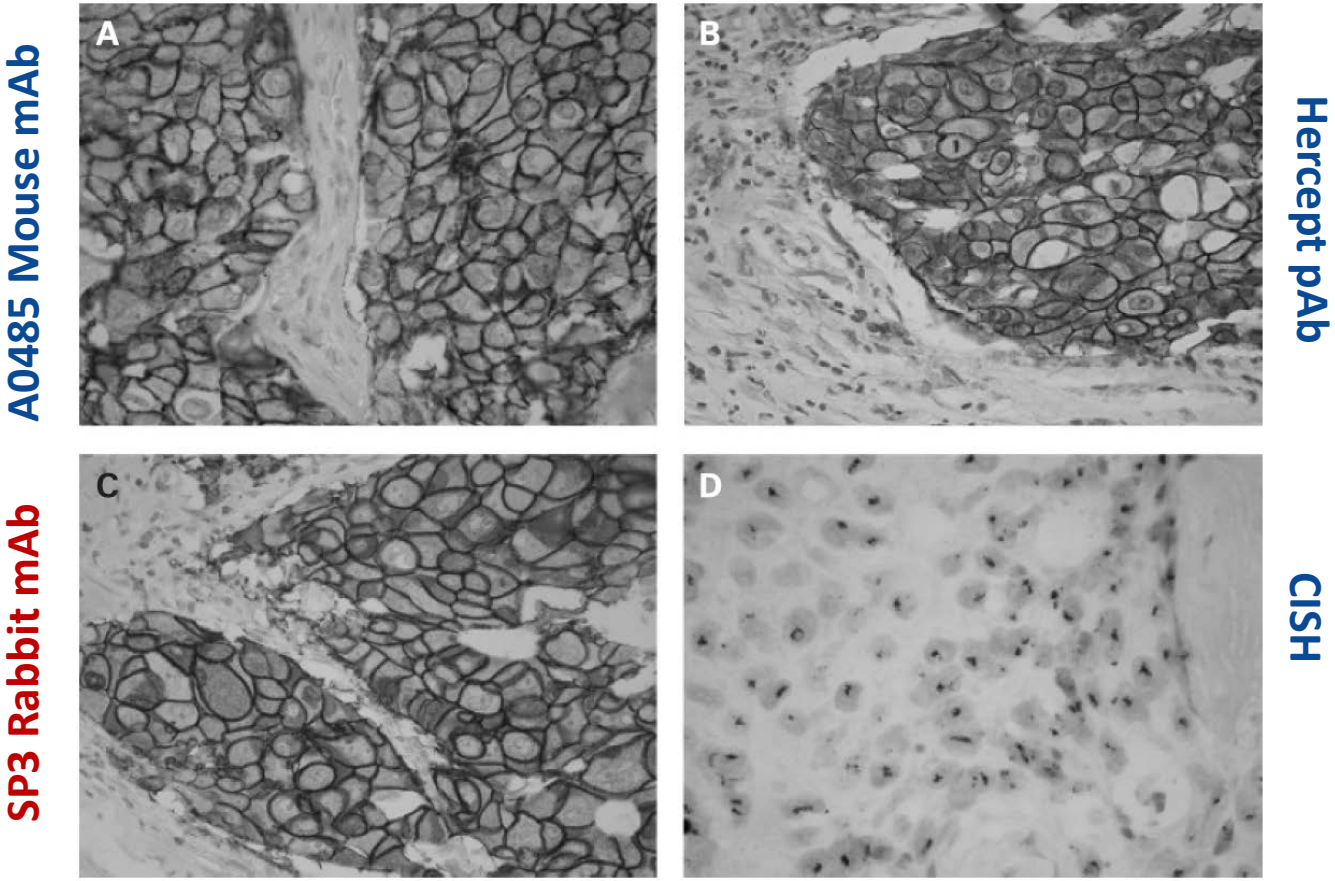
Outline

Why Rabbit mAbs are Important for IHC Application?



Breast Carcinomas & Novel HER2 Rabbit Monoclonal Antibodies

SP3 clone of rabbit mAbs: specific and showed better correlation with gene amplification by CISH



Comparative analysis of six different antibodies against Her2 including the novel rabbit monoclonal antibody (SP3) and chromogenic in situ hybridization in breast carcinomas; C B Nunes, H Gobbi; J Clin Pathol 2008;61:934–938

Rabbit Monoclonal SP3: More Sensitive to Detect HER2 than Others

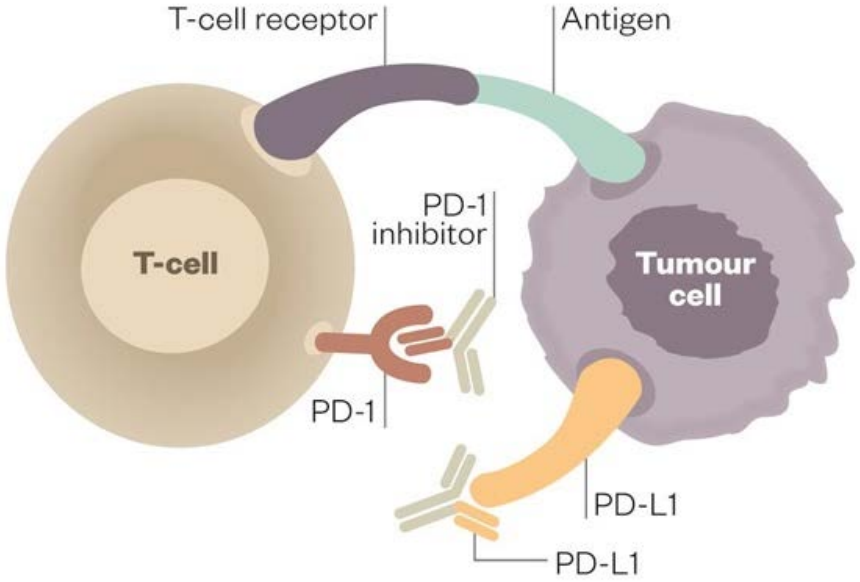
Table 2 Comparison of Her2 overexpression using six different antibodies in 84 breast cancers

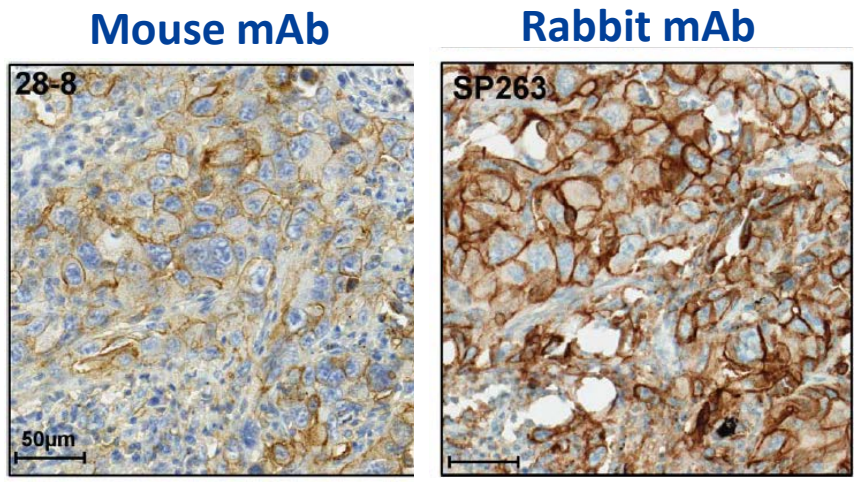
	Her2 antibodies					
IHC score	SP3	A0485	HercepTest	NCL-CB11	CM-CB11	4D5
0	14 (16.7%)	10 (11.9%)	27 (32.1%)	37 (44.0%)	32 (38.1%)	33 (39.3%)
1+	9 (10.7%)	8 (9.5%)	6 (7.1%)	2 (2.4%)	7 (8.3%)	4 (4.8%)
2+	20 (23.8%)	13 (15.5%)	8 (9.5%)	10 (11.9%)	13 (15.5%)	11 (13.1%)
3+	41 (48.8%)	53 (63.1%)	43 (51.2%)	35 (41.7%)	32 (38.1%)	36 (42.8%)

NCL, Novocastra; CM, Cell Marque.

- SP3 mAbs: specific and showed better correlation with gene amplification by CISH
- SP3 mAbs: sensitive and showed more 3+ and 2+ cases not amplified by CISH
- The correlation between CISH amplification with 3+ tumors was high whereas with 2+ tumors was indeterminate.
- CISH identified HER2 gene amplification in 46 tumors (54.8%) only

PDL-1 IHC Rabbit mAbs in Pulmonary Squamous-Cell & Adenocarcinomas





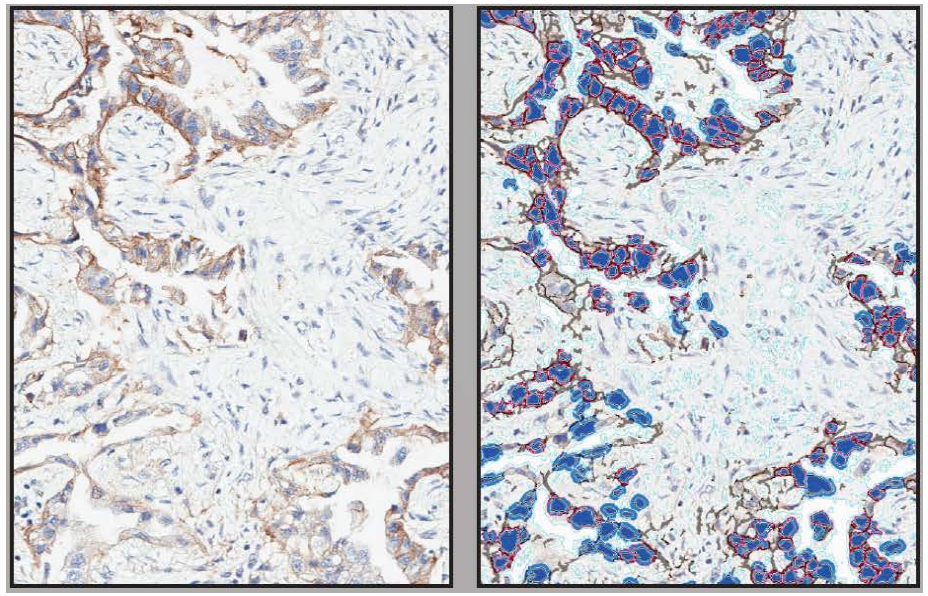
Three assays showed a linear membranous staining of the carcinoma cells (28-8, 22C3, SP263), one assay showed a membranous, partially linear, partially granular staining (SP142).

Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas Andreas H Scheel and Reinhard Buettner; Modern Pathology (2016) 29, 1165–1172

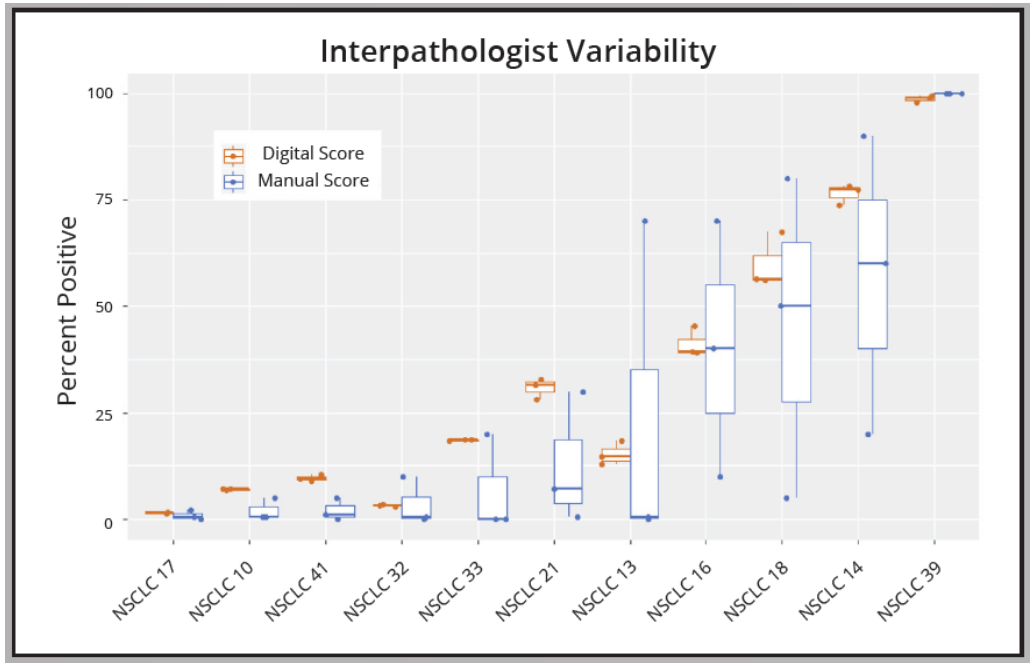
Digital Pathology in Tissue Diagnostic Applications

- Improves precision in the scoring of a challenging biomarker stain such as PD-L1
- Captures the full diagnostic spectrum of PD-L1 scoring and positivity for PD-L1 that have a low IHC staining

PD-L1 IHC Assay **cTA Markup**



cTA platform reduces variability in PD-L1 scoring



Evaluating image analysis approchaes toward “harmonization of PD-L1 assays; Flagship Biosciences

Outline

GenScript's Antibody Services

- Antibody Services
  - Antibody Drug Discovery (ADD)
    - Therapeutic Antibody Discovery Platform
    - Anti-Drug Antibody (ADA)
    - Surrogate Antibody
    - Immunogenicity Assay
  - Reagent Antibody
    - PolyExpress™ Polyclonal Ab (45 days)
    - MonoExpress™ Monoclonal Ab (45 days)
    - Phospho-Specific / Isoform Specific Antibody
    - Neutralizing and Blocking Antibody
    - Antibody Pairing
  - Catalog Antibody
  - Diagnostic Antibody
  - Immunoassay Development

Polyclonal Antibody Services

	Standard		Fully Custom	
Immunization schedule	Express (3-4 wk)	Conventional (8-10 wk)	Express (3-4 wk)	Conventional (8-10 wk)
Host	Rabbit	Rabbit/Rodent	Rabbit	Rabbit/Rodent
Antigen	Peptide/protein		Peptide/protein	
Antiserum delivery during the process	N/A		Yes	
Final delivery	Purified antibody		Serum purified antibody	

PolyExpress™ Custom pAb Packages

Protein antigen

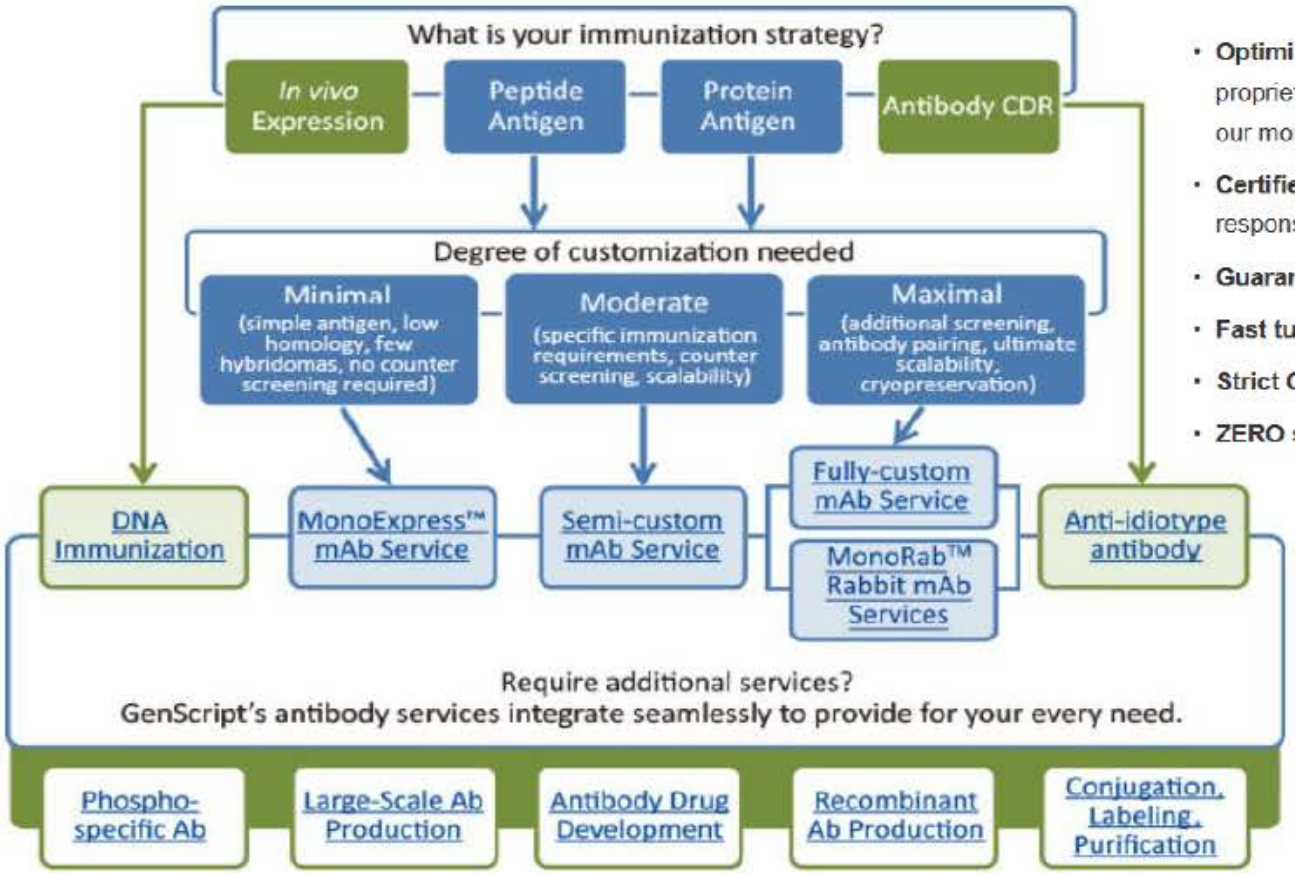
PolyExpress™ **Premium**	PolyExpress™ **Premium Plus**
Use recombinant protein as antigen**	
2 affinity-purified pAb (1-6 mg each) and 200 ug protein antigen	**1 affinity-purified pAb (≥2 mg) and 1mg protein antigen**
Guaranteed to perform in antigen **ELISA and WB** applications	Guaranteed to perform in antigen **ELISA**, **WB**, and **IP** applications

Peptide antigen

PolyExpress™ **Basic**	PolyExpress™ **Silver**	PolyExpress™ **Gold**
Uses 1 peptide to make antibody	Uses 2 peptides to make antibodies	Uses 3 peptides to make antibodies
1 Ab generated against 1 epitope	2 Abs generated against 2 individual epitopes	3 Abs generated against 3 individual epitopes
Guaranteed ≥2 mg purified antibody	2 affinity-purified antibodies (≥2 mg)	3 affinity-purified antibodies (≥2 mg)
Application Success Rate		

- **Cost effective and comprehensive:** pricing starts from $649 and includes antigen synthesis and purification!
- **Fast turnaround time:** delivery of purified custom polyclonal antibodies in as **little as 45 days**.
- **Optimal immunization**: Our OptimumAntigen™ design tool and intelligent Antigen Strategy increase specificity and affinity of antibodies.
- **Guaranteed results:** affinity purified custom polyclonal antibody quantities and ELISA titers of 1:64,000.
- **Certified facility:** AAALAC International accreditation and OLAW certification, demonstrating our commitment to responsible animal care and use.

Customized mAb Service



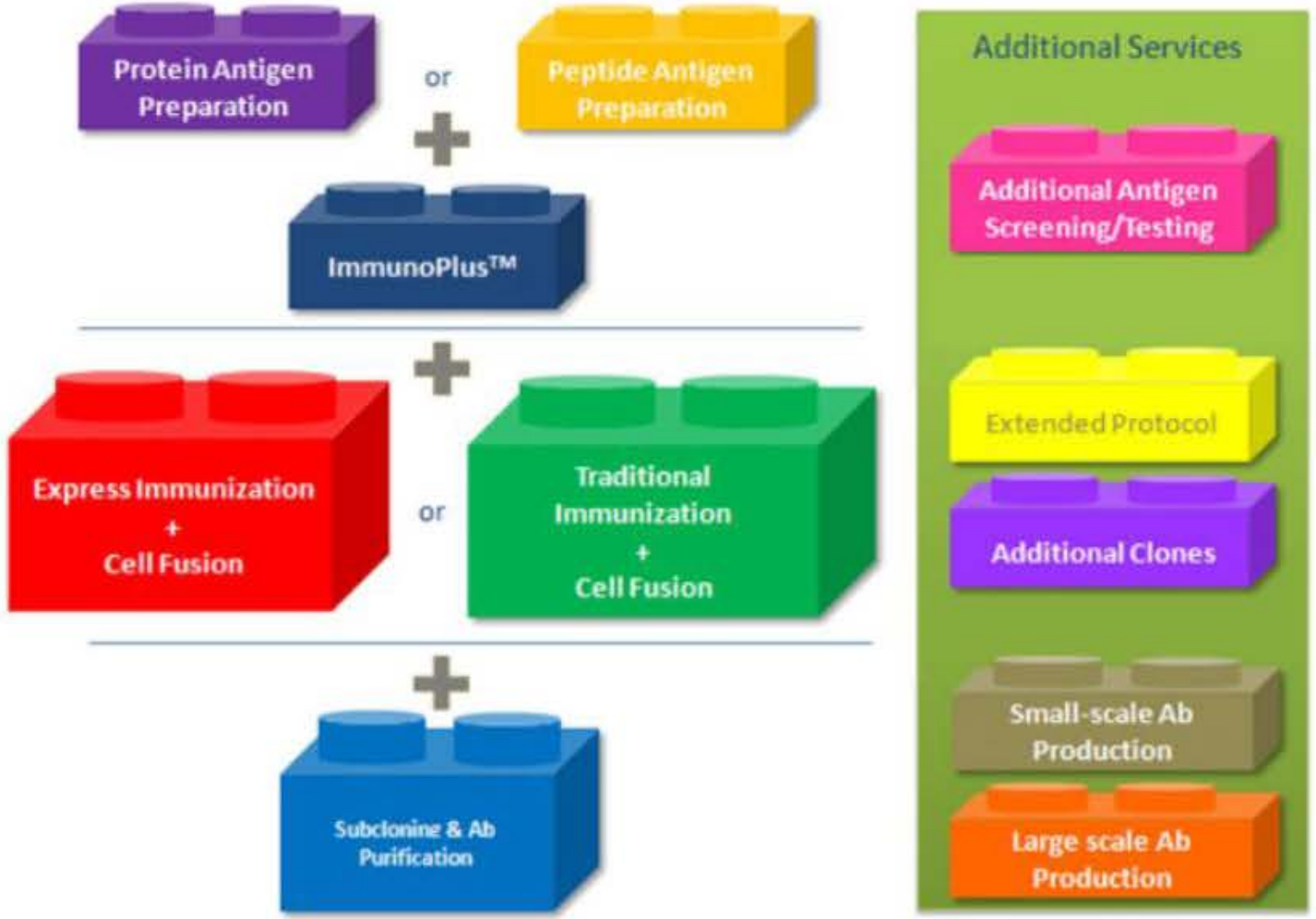
- **Optimized immunization**: Our advanced technologies - OptimumAntigen™ design tool, intelligent Antigen Strategy, proprietary immunization technology, and optional GANP® mouse technology - increase the specificity and affinity of our monoclonal antibodies.
- **Certified facility**: AAALAC International accreditation and OLAW certification, demonstrating our commitment to responsible animal care and use.
- **Guaranteed results**: ELISA titer and Western blot guaranteed packages available.
- **Fast turnaround time**: Delivery of monoclonal antibodies 3 weeks faster than our competitors.
- **Strict Confidentiality**: No project associated information and products will be disclosed except to the project owner.
- **ZERO setup fee**: Our confidence and near 100% success rate limit the risk to you.

MonoExpress

Service	Antigen	Immunization	Customer Screening	Deliverable	Timeline
Premium	Protein produced at GenScript	Mice/rats	ELISA with 10 supernatants & Supernatants for in house analysis	5 hybridoma cell lines, 2mg purified mAb from 1 hybridoma, & 200ug immunogen	11 Weeks
Basic	Provided protein	Mice/rats	ELISA with 5 supernatants & Supernatants for in house analysis	5 hybridoma cell lines,& 2mg purified mAb from 1 hybridoma,	6-7 Weeks
Gold	3 Genscript generated peptides	15 Mice/rats	N/A	6 hybridoma cell lines, 6 supernatants (5 ml/clone), 2 mg unconjugated peptide	13 weeks
Silver	2 Genscript generated peptides	10 Mice/rats	N/A	4 hybridoma cell lines, 4 supernatants, 2 mg unconjugated peptide	13 weeks
Bronze	1 Genscript generated peptide	5 Mice/rats	n>/A	2 hybridoma cell lines, 2 supernatants, 2 mg unconjugated peptide	13 weeks

Semi Custom mAb Services

Semi-custom Monoclonal Antibody Production Services Diagram



Fully Custom mAb Services

Phase	Service Options	Deliverables	*Mid-project Phase-specific Options	Approximate Time (weeks)
Phase I **Immunization (SC1216)**	•MonoExpress™ protocol or conventional protocol (4 months longer) •ImmunoPlus™ Technology •DNA Immunization •Animals: BALB/c mice, C57BL6 mice, or rats •Number of animals immunized	•Test bleed report or 15 ul serum samples after 3rd boost	•Modify immunization strategy. •Proceed with selected animals for fusion.	8-10
Phase II **Cell Fusion and Screening (SC1219)**	•Indirect ELISA screening and WB validation •Capture ELISA •Competitive ELISA •FACS screening •Counter screening •Additional screening options available	•Parental supernatant report or 2 mL supernatants	•Alter screening methodology. •Select ~5-10 clones which proceed to subcloning.	5-7
Phase III **Subcloning, Screening, and Expansion (SC1220)**	•Subclone Screening •Cryopreservation •Cell banking •Roller Bottle Production •Antibody Purification	•2 vials of up to 10 hybridoma cell lines •5 mL sub-clone supernatant •Purified Antibodies	•**FREE** cell banking at GenScript for 6 months. •Scale-up production •Continue with other services: antibody screening, rAb production, etc.	4-7

MonoRab™
GenScript's Premier Rabbit Monoclonal Antibody Generation Service

WEBINAR

Workflow

Milestone	Details	Times (weeks)
Immunogen preparation	• Antigen production • KLH conjugation for peptide Ag	3
Animal Immunization	• New Zealand White Rabbit • 2 Animals/group • # groups optional	10-12
Cell Fusion, Screening, Subcloning & Ab Sequencing	• 1 Electro fusion of up to 150 96- well plates • ELISA supernatant screening • Subcloning • V-region sequencing of up to 10 monoclones • Up to 10 parental clones selected for customer analysis • Up to 5 clones selected by Client	10-11
FREE Epitope Binning	• Free epitope binning service included in every MonoRab™ purchase • Service includes epitope binning on 5 monoRab™ chosen after in house screening	0.5
Optional Recombinant Ab Production	• Up to 5 clones selected by client • Ab expression and purification from plasmid • QC with ELISA, SDS-PAGE, & OD280nm	4-5

Exceptional Specificity & Sensitivity

Extremely Fast Turn Around Time

Most Cost Effective Pricing

Application Tailored Services

Anti-Idiotype mAb Generation
Superior Sensitivity To Detect Minimal Traces of Antibody Drug

IHC Tailored mAb Generation
Extraordinary Specificity Resulting in Nominal Background Levels

ScFv generation
Strong Affinity Allowing For Production of High Quality mAb Fragments

Summary

- Rabbit mAbs have a significantly higher affinity, specificity and sensitivity.
- Rabbits have diverse antibody repertoire, greater response to small antigens.
- Several technologies are available to generate rabbit mAbs: hybridoma, phage display and single B-cell cloning.
- Usage of rabbit mAbs are increasing in tissue diagnostics and immunochemistry to provide precision diagnosis for patients treatment.
- Digital pathology quantitative analysis may provide accurate and reproducible data, same as pathologists'
- GenScript provides one-stop solution for antibody production.

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