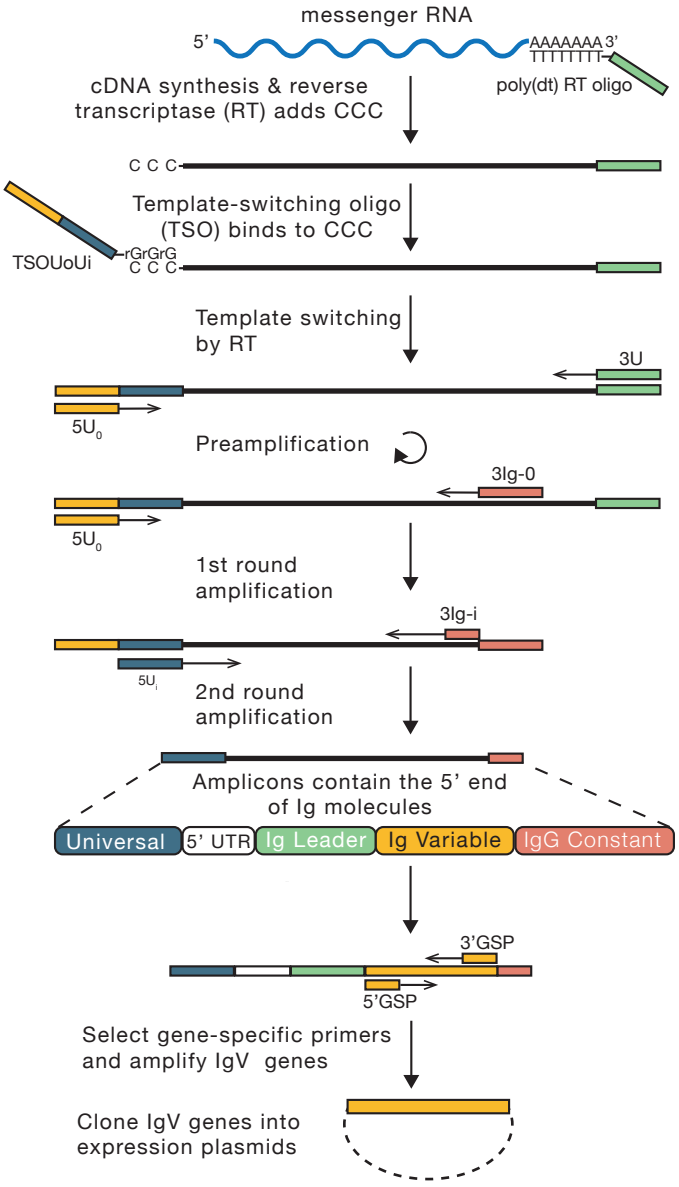
Monoclonal Antibody Isolation Service

Summary

We offer a cutting-edge service for isolating monoclonal antibodies (mAbs) from individual B cells using our novel SMART-RACE-based approach[1]. Unlike traditional PCR-based methods, which often struggle to amplify diverse or poorly characterized immunoglobulin genes, our method eliminates the need for complex multiplex primer sets, simplifying amplification and improving recovery rates from individual B cells. Initially validated in rhesus macaques, our versatile method is adaptable to virtually any species, making it an ideal platform for researchers isolating novel antibody reagents or therapeutic mAbs from diverse species, including mice, primates (macaques, humans) and non-traditional animal models (ferrets, hamsters, dogs, pigs).



Steps for cloning antibodies from individual B cells by 5'SMART-RACE. Our approach appends synthetic primer binding sites to both ends of the cDNA during synthesis. These universal primer binding sites enable amplification of low-abundance transcripts in memory B cells and eliminate the need for species-specific leader sequence primers. This design allows for the efficient amplification of imunoglobulin genes across diverse species.

Our approach processes up to 96 individual B cells simultaneously, from cell sorting through sequencing to recombinant antibody production. This scalable workflow accommodates everything from small pilot studies to large-scale antibody discovery campaigns.

Workflow

- **Samples**: We accept peripheral blood mononuclear cells (PBMCs), sorted B cells, or other appropriate tissue samples.
- **Antigen-Specific B Cell Isolation**: Individual B cells recognizing your antigen of interest are isolated using fluorescence-activated cell sorting (FACS).
- **SMART-RACE cDNA Synthesis**: Optimized reverse transcription with template switching generates full-length cDNA with universal primer binding sites.
- **Nested PCR Amplification**: Our universal 5' primers, paired with constant region-specific 3' primers, efficiently amplify immunoglobulin variable regions.
- **Sequence Analysis**: High-quality sequences are analyzed to identify paired heavy/light chains and to select gene-specific primers for antibody cloning.
- **Antibody Expression**: Recombinant antibody production in mammalian cell lines, with functional validation available.

Potential Applications

- **Resource Development**: Isolate mAbs against antigens of interest for novel research tools.

- **Vaccines**: Characterize B cell responses to candidate vaccines in pre-clinical models.
- **Infectious Disease Research**: Isolate pathogen-specific neutralizing antibodies.
- **Non-Traditional Animal Models**: Investigate specific antibody responses in species such as ferrets, hamsters, dogs, and livestock.
- **Therapeutic Antibody Discovery**: Identify novel antibody candidates from antigen-exposed individuals.

Deliverables

- DNA sequences of immunoglobulin variable regions
- Sequence analysis report including
  - —V(D)J gene usage and allele identification
  - —CDR length and amino acid sequences
  - —Somatic hypermutation analysis
- Recombinant antibody expression vectors
- Purified recombinant antibodies (mg quantities)
- Antigen-binding validation by ELISA and/or flow cytometry

Getting Started

Contact Dr. Matt Reynolds (matt.reynolds@wisc.edu) to discuss your project requirements. We offer customizable service packages, ranging from simple antibody sequence recovery to complete workflows, including B cell sorting, sequence analysis, recombinant antibody production, and antigen-binding characterization.

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[1]Our SMART-RACE technology is based on methods described in Weinfurter et al. (2024), Journal of Immunological Methods, 525, 113602. https://doi.org/10.1016/j.jim.2023.113602