Sequencing Existing Antibodies

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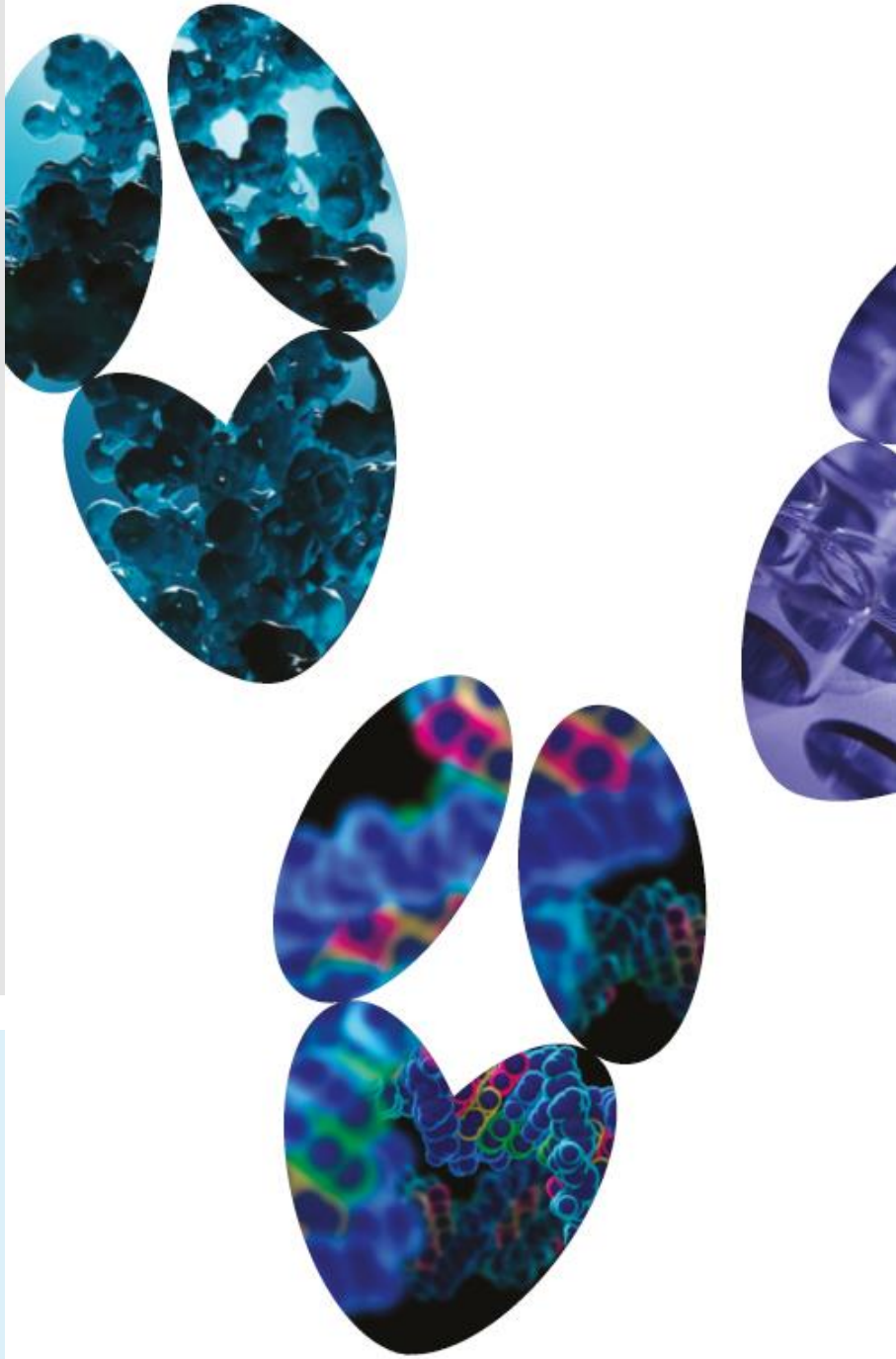
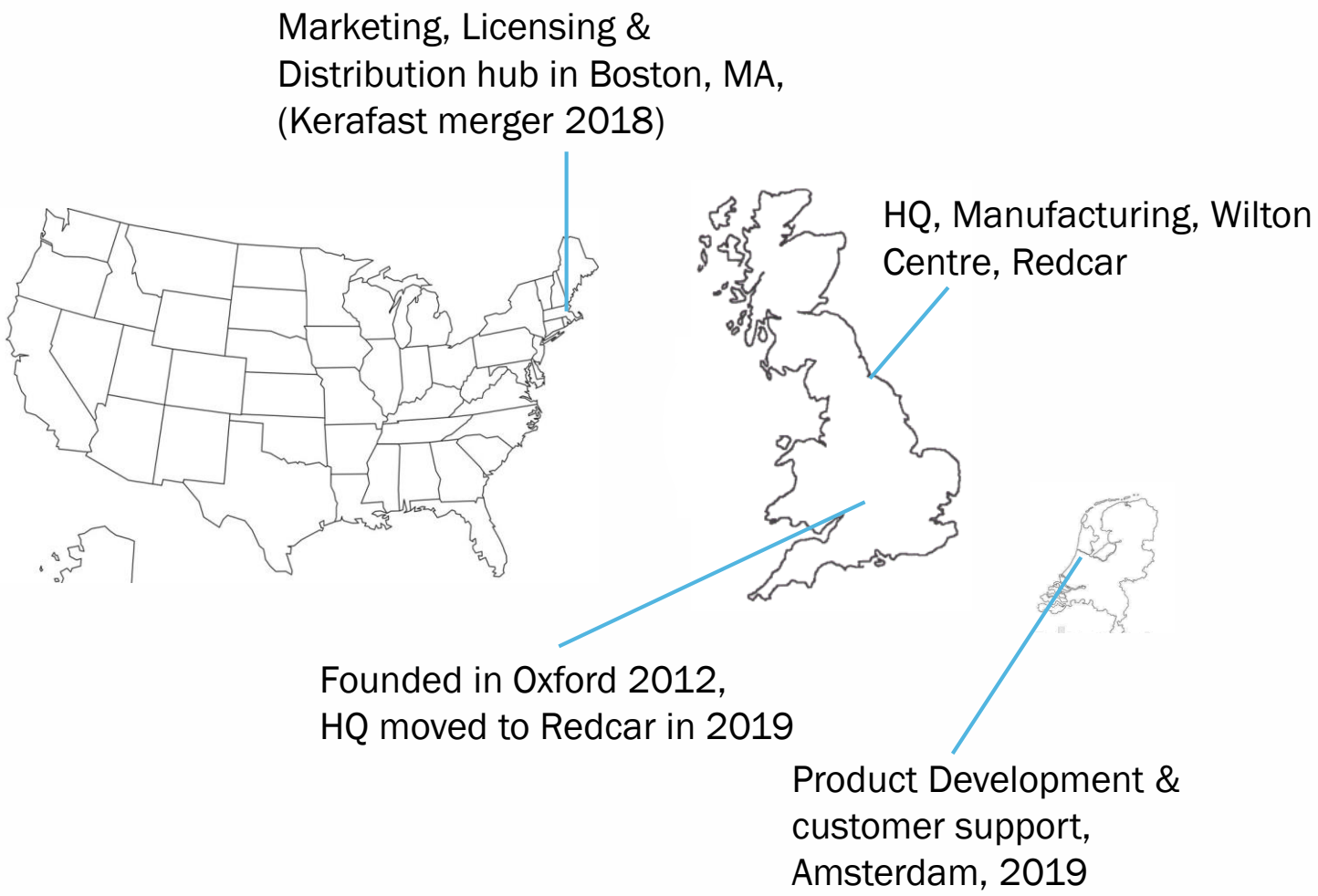
absolute antibody



Company Overview

- Antibody supplier and CRO
- Founded in 2012, rapidly growing, currently ~60 staff across 3 locations
- Company vision

  To make recombinant engineered antibodies and related services more accessible to the wider community, particularly those in research and diagnostics.
- Industrialized 3 core technologies applied to services and catalogue
  - Hybridoma sequencing
  - Antibody engineering
  - Transient expression



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Reagents for the Greater Good

What is a hybridoma – and why are we falling out of love?

- Fusion of an antibody-secreting B-cell from an immunized animal with a myeloma cell
- Immortalized antibody-producing cell line you can culture *in vitro*

- Genetic make up a fusion of the B-cell & myeloma cell
- Genetic instability
- Transcript contribution from myeloma (incl. antibody transcripts)
- Multiple fusion events
- Sometimes difficult to adapt to serum-free growth
- Sometimes no *in vitro* growth, requiring ascites production
- Clones can die off or be lost

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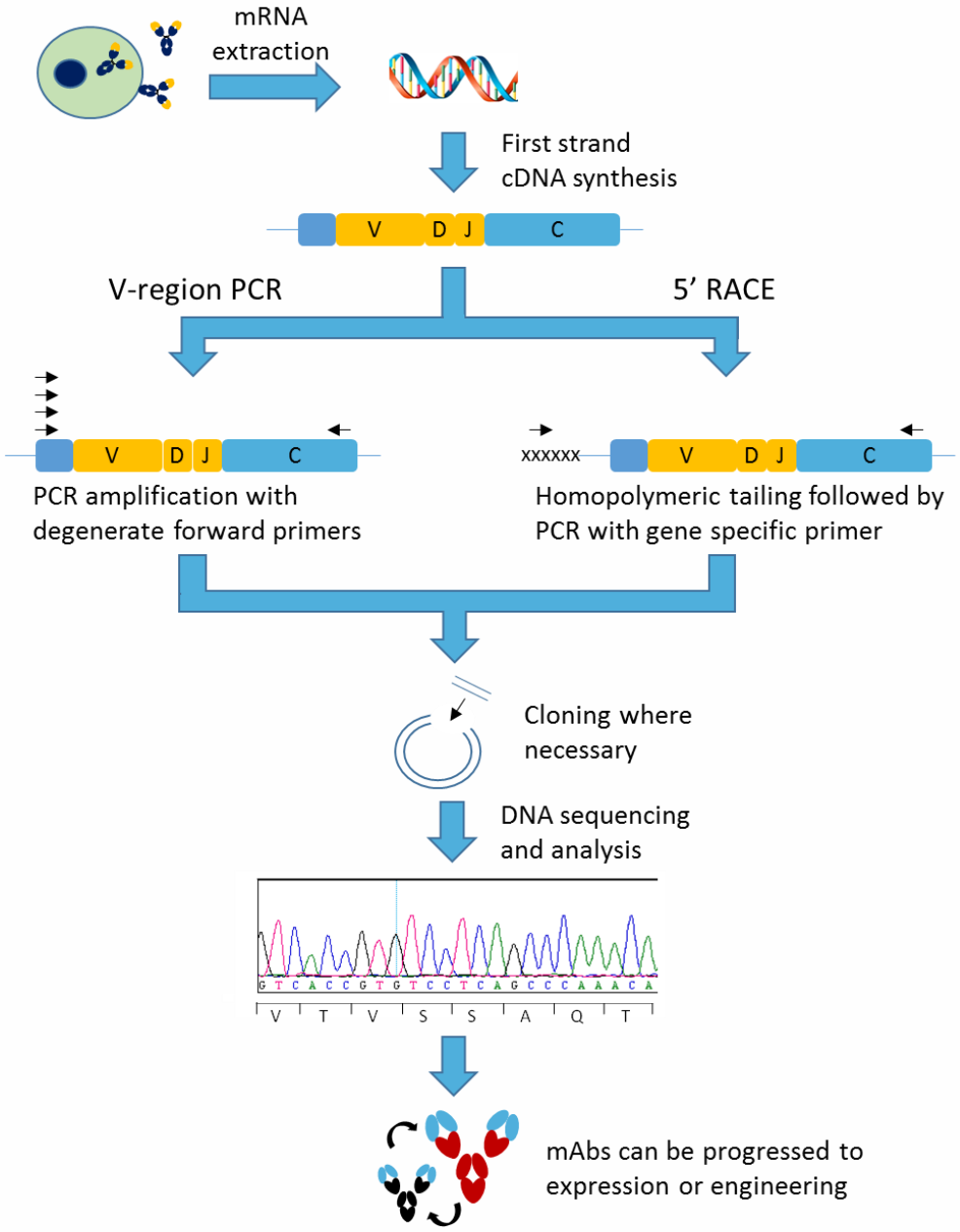
Hybridoma sequencing

- Hybridoma sequencing techniques first developed in the 1980s:
  - V-region PCR
  - 5’ RACE (rapid amplification of cDNA ends)
- Vast majority of people sequencing hybridomas still use these techniques
- Advantages:
  - Low consumables cost
  - Relatively simple
- Disadvantages:
  - Low throughput, requires lots of hand-on time, not easy to automate
  - Error prone
  - Difficult to sequence non-monoclonal hybridomas



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“Not-so-mono” monoclonals

- Study of 187 contained or
- Switching to performance genes
- “When monc Hybridomas variable regi

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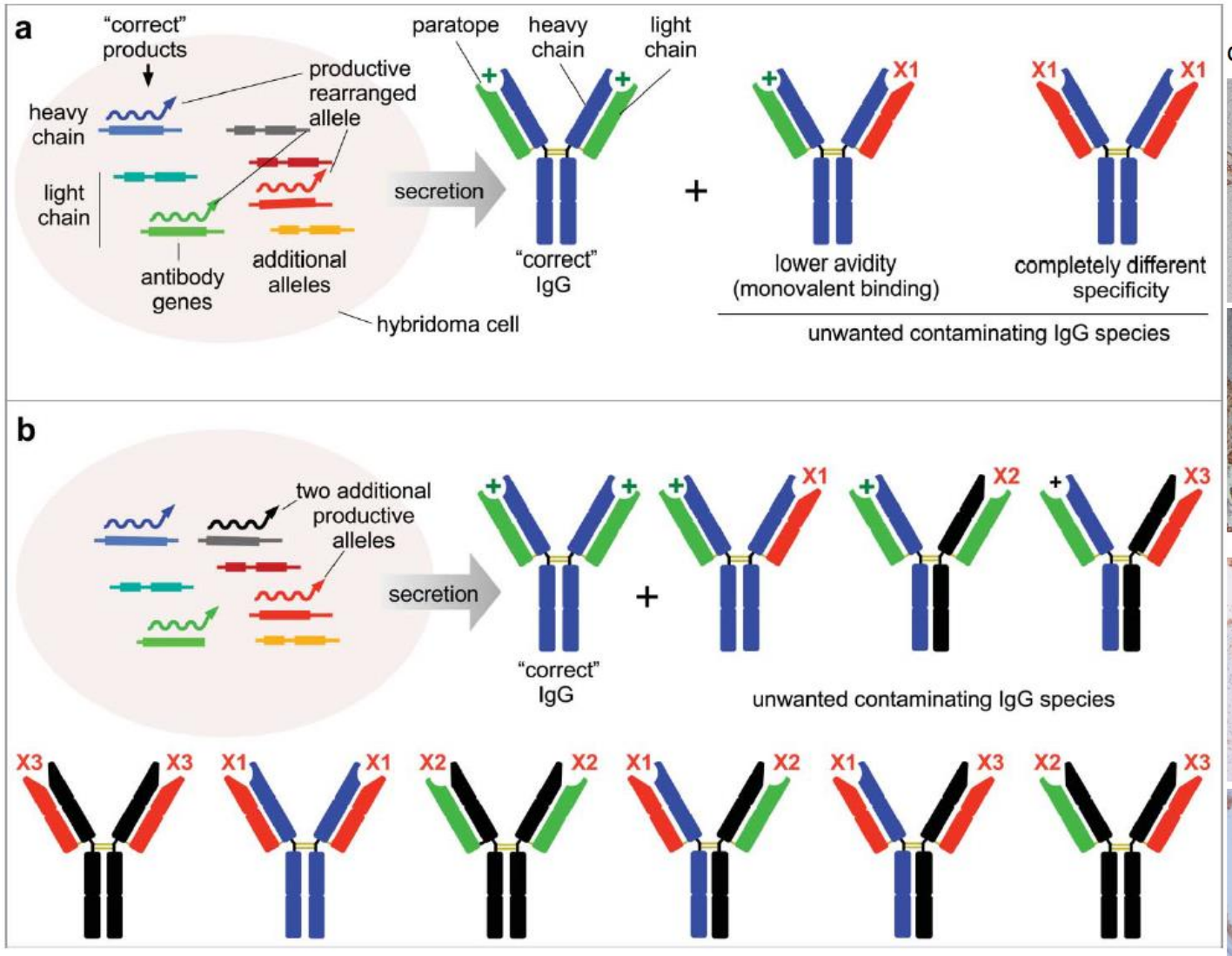

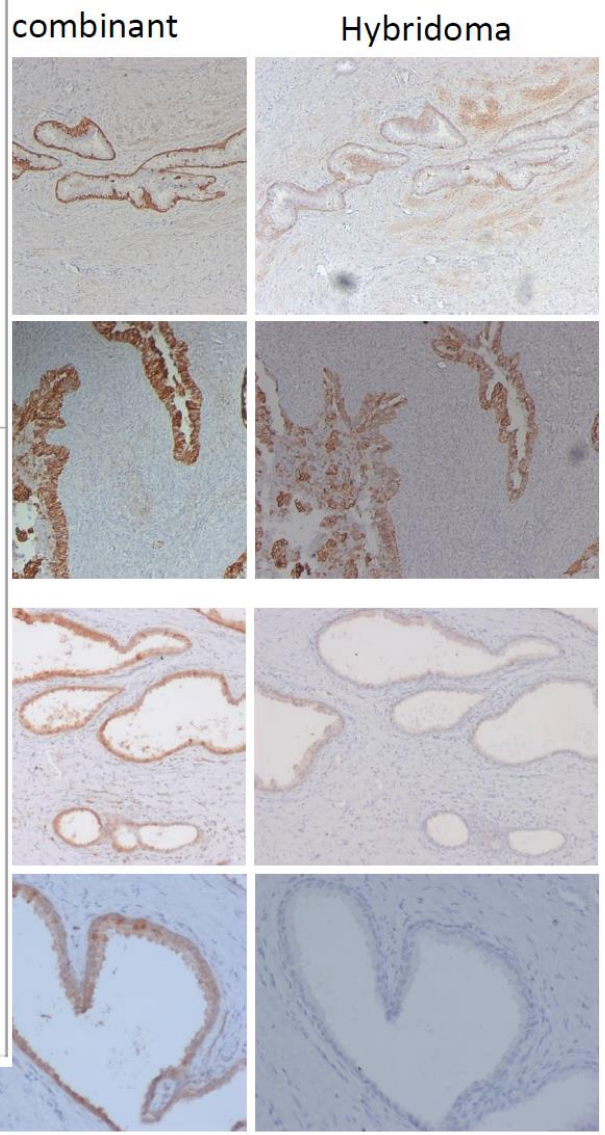

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Sequencing Problems

- No correlation of abundance with functionality
  - The “right” transcript may be 1:100 or 1:1000!
- Not always identifiable as not always a “known” aberrant transcript
- Focus on V-regions omits information in constant domains
- Does it matter? Does it really cause problems?
  - Public sequence data bases contain many artefacts (Patents, Papers, Crystal Structures)
  - Well-known myeloma-derived sequences
  - Non-functional sequences, non-expressing sequences

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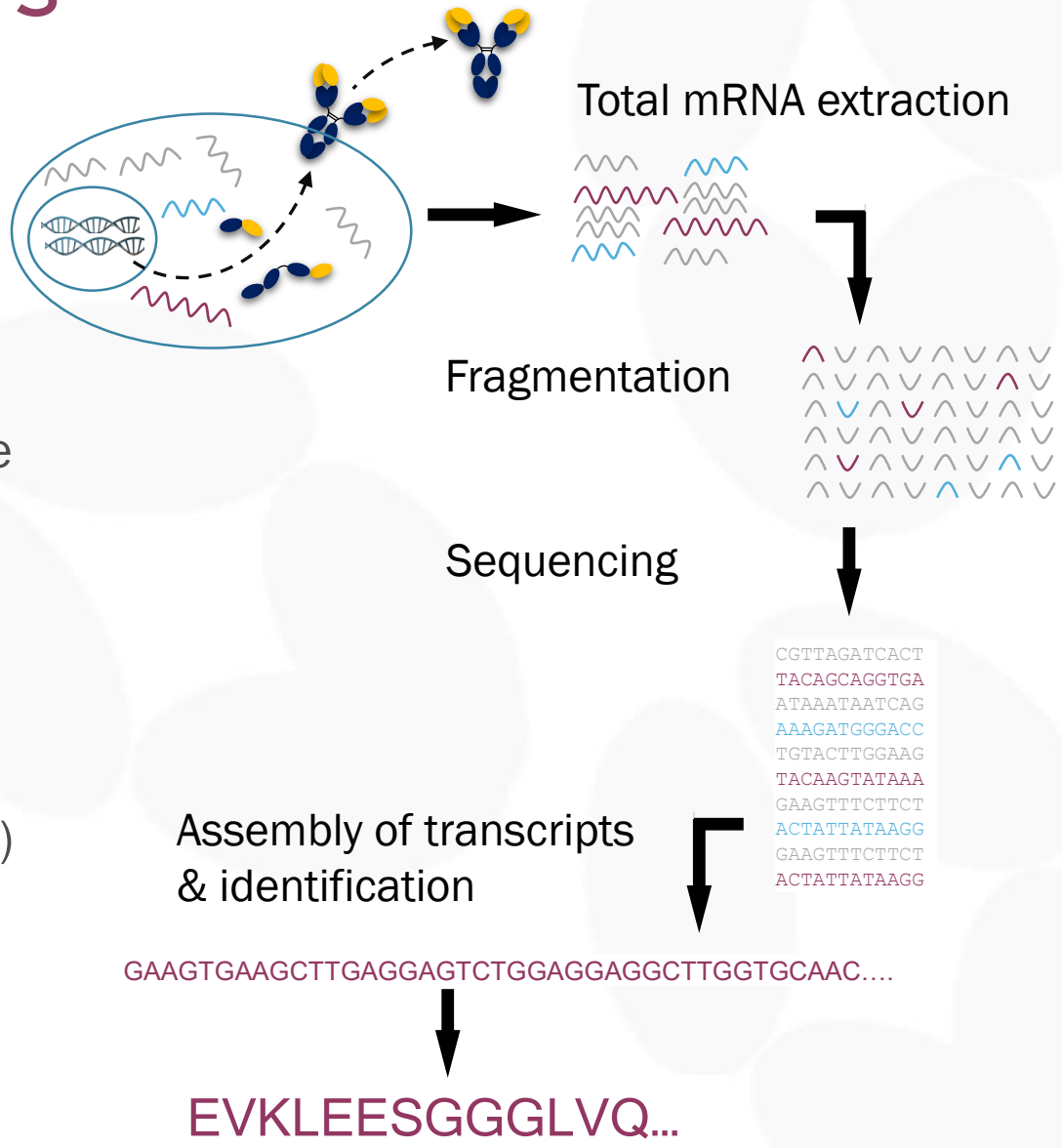
Hybridoma Sequencing – next generation

- Developed a new NGS based approach called Whole Transcriptome Shotgun Sequencing
  - No specific primers required
  - Compatible with any species and isotype
  - High throughput
  - Fully automated data analysis
  - Identifies all antibody related transcript, even those at very low mRNA abundance
  - Rescue “dead” hybridomas
  - Able to identify full antibody sequence
- Approaches combining NGS with RACE-PCR?
  - Lower sensitivity
  - Focused on certain species (mouse, human, rabbit) and isotypes
  - Need to know species and isotype of starting antibody before sequencing
  - Only identifies variable domain sequences



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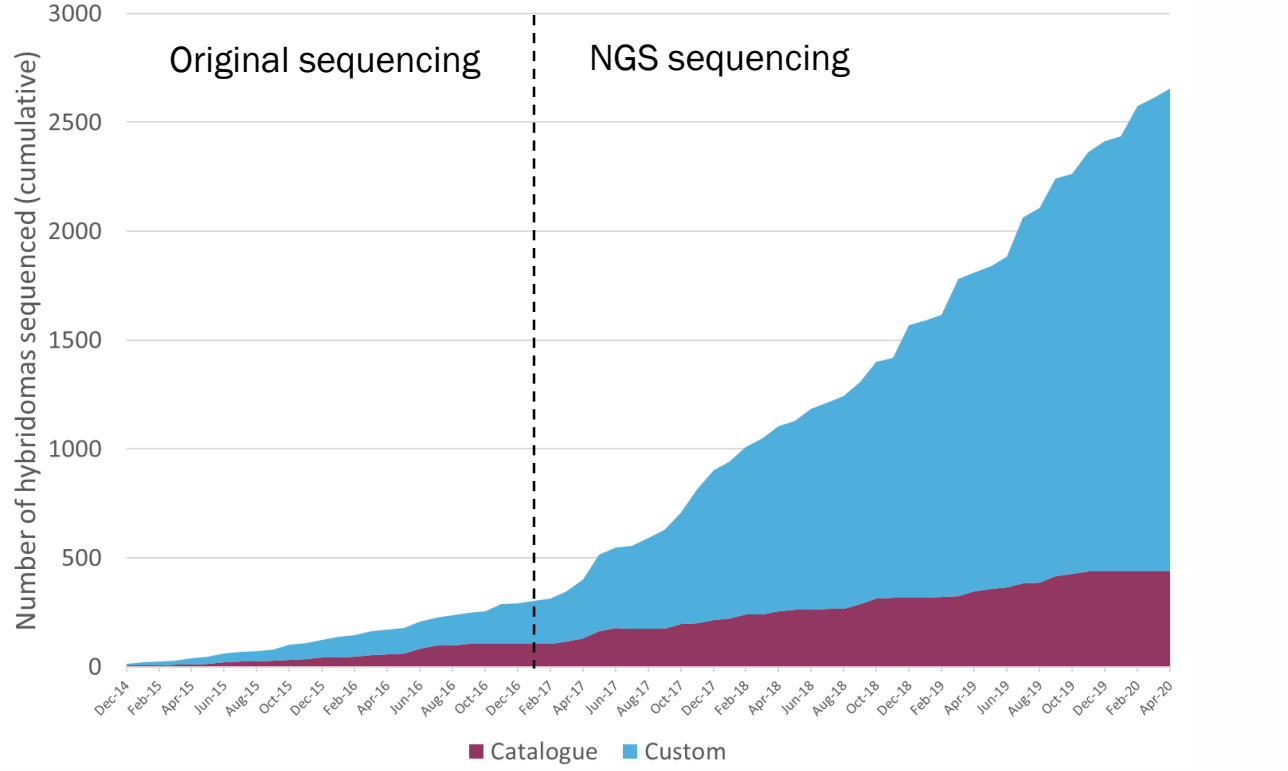
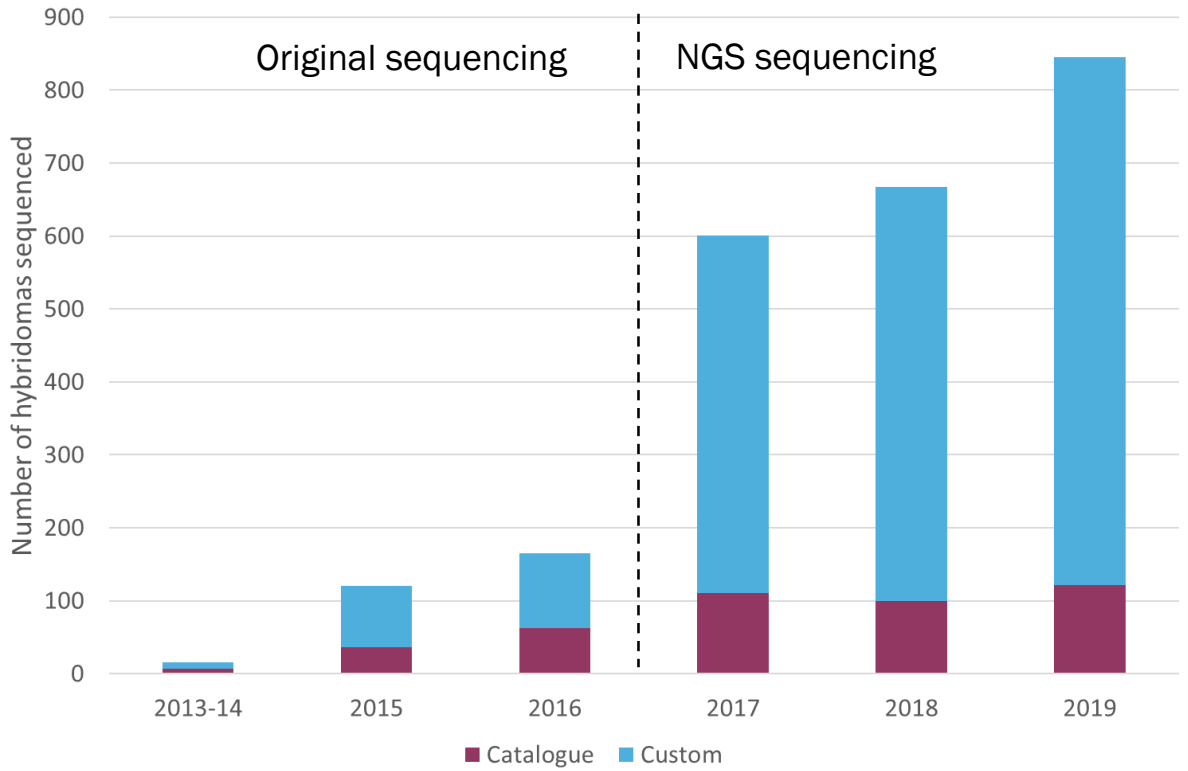
Vorsprung durch Technik





- Before we launched NGS sequencing we struggled to sequence samples quickly enough. Approx. 300 clones in 4 years
- Since launching NGS sequencing our bottleneck has feeding the pipeline. Theoretical sequencing capacity is +10,000 samples per year.
- Advantages: any species, highest possible quality, full sequences
- Disadvantages: slow (for small sample numbers), higher operating costs

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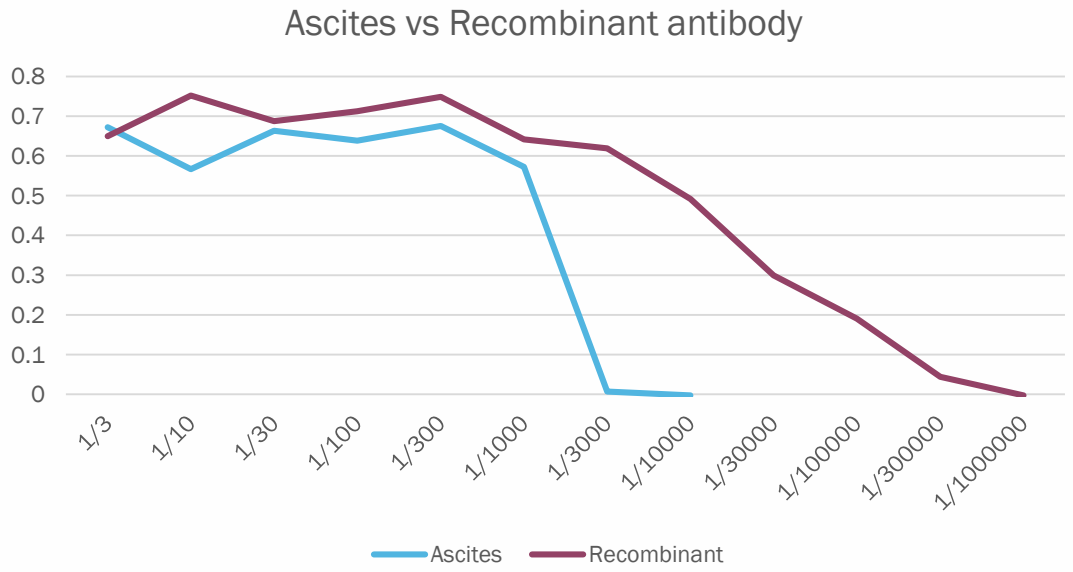
What about ascites production?

- There are clones that can only be grown in ascites
- Sequencing these clones is no different to sequencing a clone that will grow *in vitro*
- Example antibody where sequencing revealed no secondary antibody transcripts
- Recombinant version out-performs original, ascites-derived monoclonal.



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No More Excuses for Hybridomas

- Sequencing approaches are reliable and work for any hybridoma
- We have reached the throughput required to sequence every available hybridoma

- But…what about “lost clones”?

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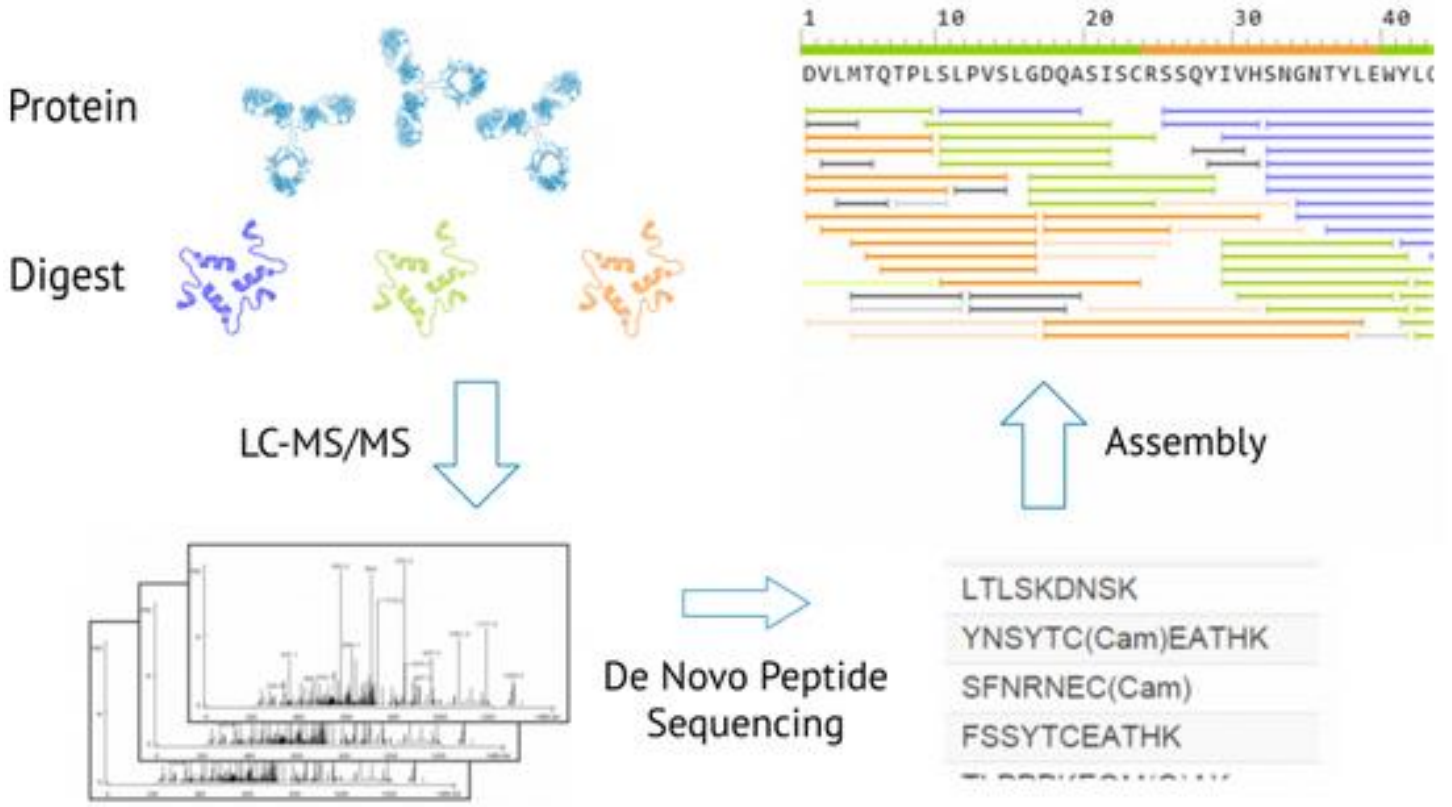
Antibody Protein Sequencing



Approx. 100 ug of pure protein required to sequence an antibody

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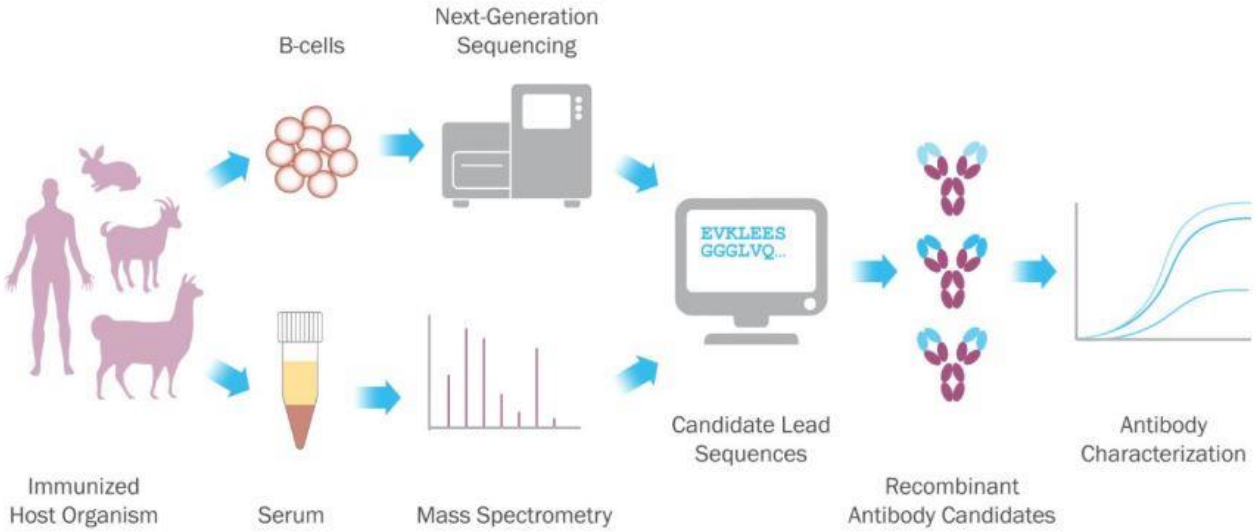
What About Valuable Polyclonals?

- There are many fantastic polyclonal antibodies out there, with performance characteristics that cannot be recapitulated by a monoclonal
- Current sequencing approaches mainly looking to combine transcriptomic and proteomic approaches



- "Holy Grail": Mass-spectrometry on polyclonal protein to identify a subset of key binders within a mixture?

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Summary

- Antibody sequencing is a good thing.
  - Quality, reproducibility, engineering options, animal welfare….
- Classic hybridoma sequencing is limited in terms of quality and throughput
- Shotgun-sequencing approaches (aka NGS) not only brings with it the quality, but also the throughput to allow us to sequence every hybridoma.
- Reliably.
- Protein sequencing approaches available for monoclonals (as a last resort?)
- Polyclonal antibody protein sequencing as a (still) missing building block in providing complete solutions for the migration of all animal-derived affinity reagents to recombinant production

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