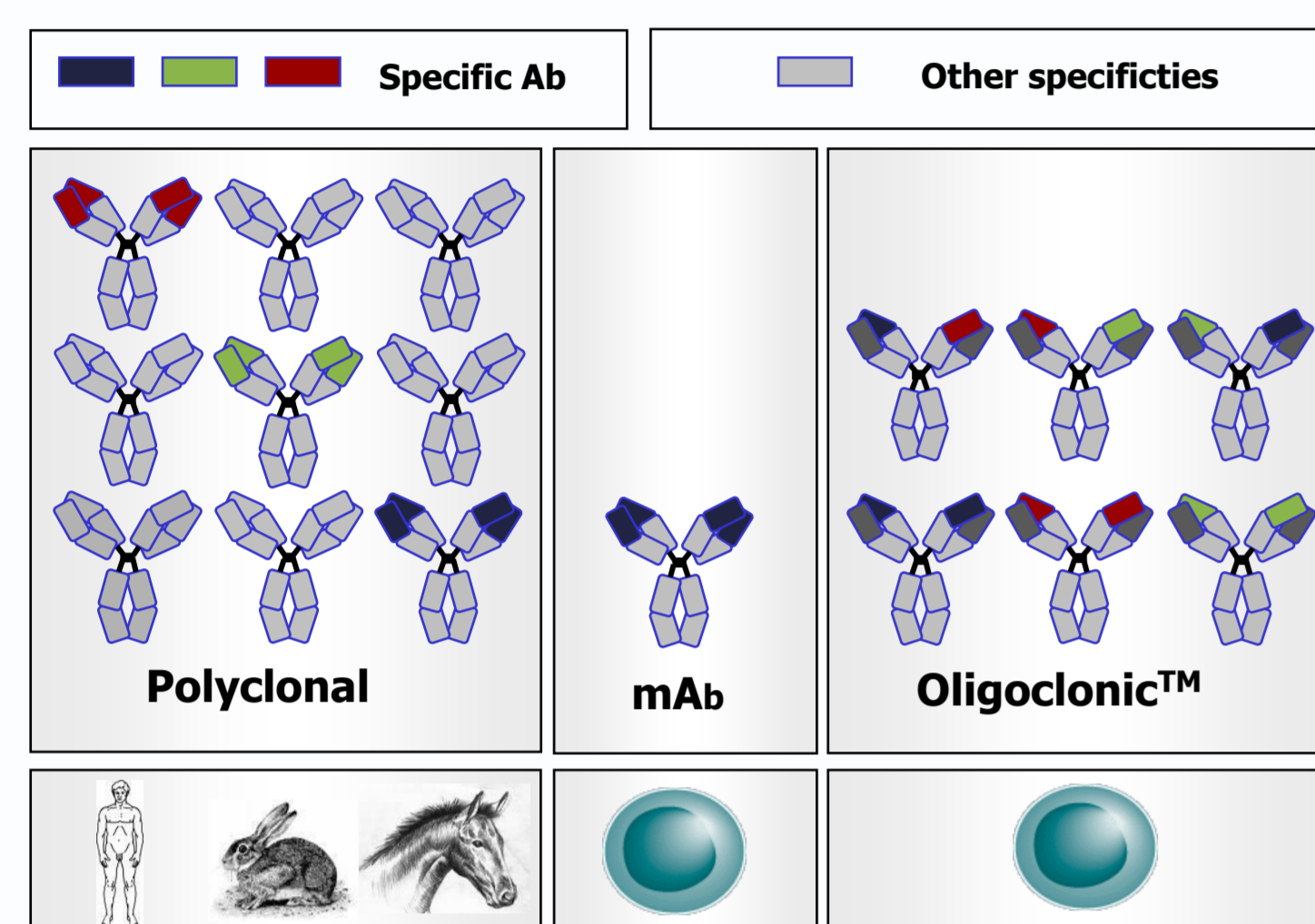


Oligoconics™



Polyclonals: cutting edge technology in the 1900's but have disadvantages of batch-to-batch variation, donor supply and contamination from blood borne infectious agents

mAbs: successfully introduced in 1987, solve many of the manufacturing issues but lack the broad coverage and synergistic potency afforded by polyclonals

Oligoconics™: combines the manufacturing advantages of mAbs with the synergy and breadth of polyclonals

Abstract

Preclinical and clinical evidence indicates that mixtures of therapeutic antibodies provide improved efficacy; however the research, manufacturing and regulatory costs of developing multiple mAbs poses a barrier to the use of antibody combination therapies. To address this issue, we developed the Oligoconics™ Technology, as a platform for the production of mixtures of mAbs by single clonal cell lines.

To avoid V_H - V_L mispairing, mAbs in an Oligoconics™-producing cell line are encoded by a single V_L region and multiple different V_H regions. For proof-of-concept of the 'single V_L approach', a phage display repertoire was constructed from a single V_L gene and V_H regions from tetanus toxoid vaccinated donors. After selections, 129 unique antibodies representing 53 unique V_H rearrangements were isolated. The antibodies were characterized by diverse V_H , D_H and J_H gene segment utilization, 17 non-competing epitope specificities and a median monovalent affinity of 2 nM.

Clonal Per.C6® cells producing Oligoconics™ were obtained by transfection with three plasmids encoding different mAbs. Batch cultures of clonal cell lines stably produced all three mAbs at high levels and in stable ratios.

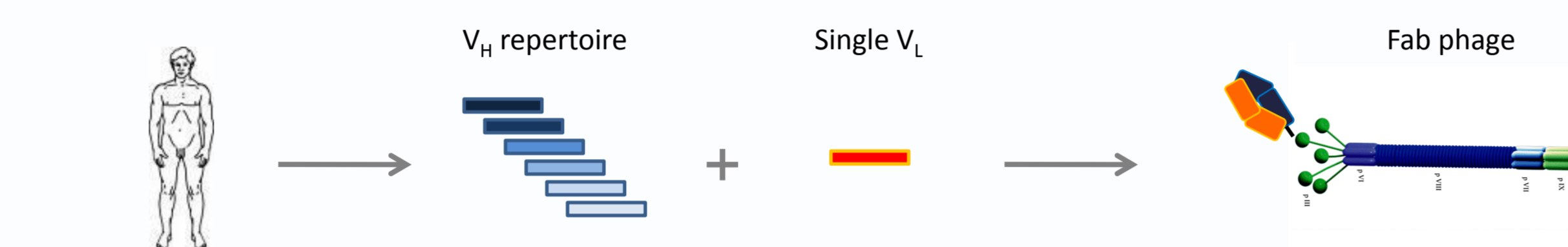
Conclusion

We conclude that the Oligoconics™ Technology yields stable cell lines expressing multiple antibodies and that 'single V_L ' mAbs suitable for Oligoconics™ are readily identified.

- References**
1. J. Mol. Biol. (2006) 358, 764-772
2. J. Immunol. (2007) 179, 3841-3850
3. Eur. J. Immunol. (2005) 35, 1231-1245
4. J. Virol. (2006) 80, 6982-6992

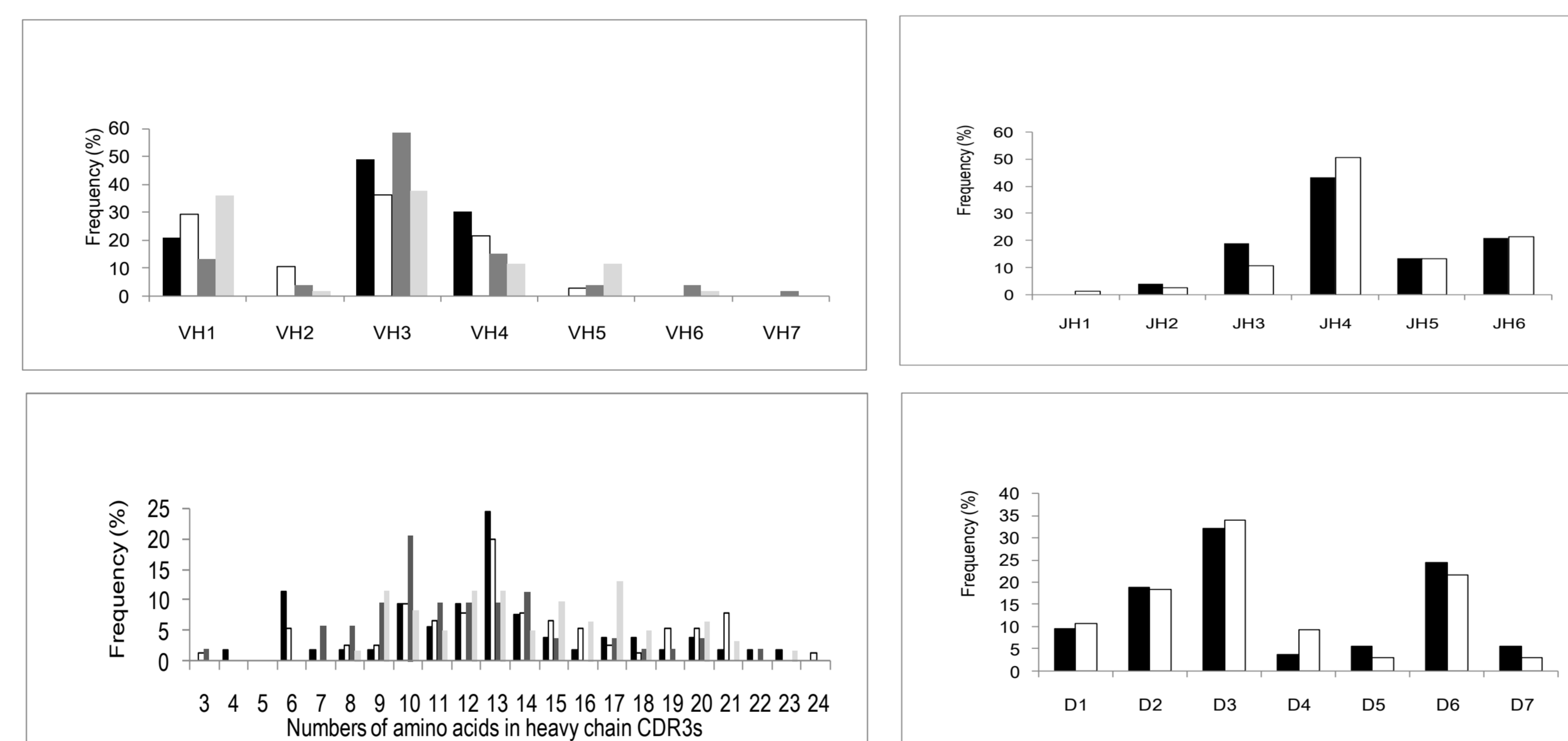
Oligoconics™ Single V_L Discovery

Single V_L repertoire discovery using phage display



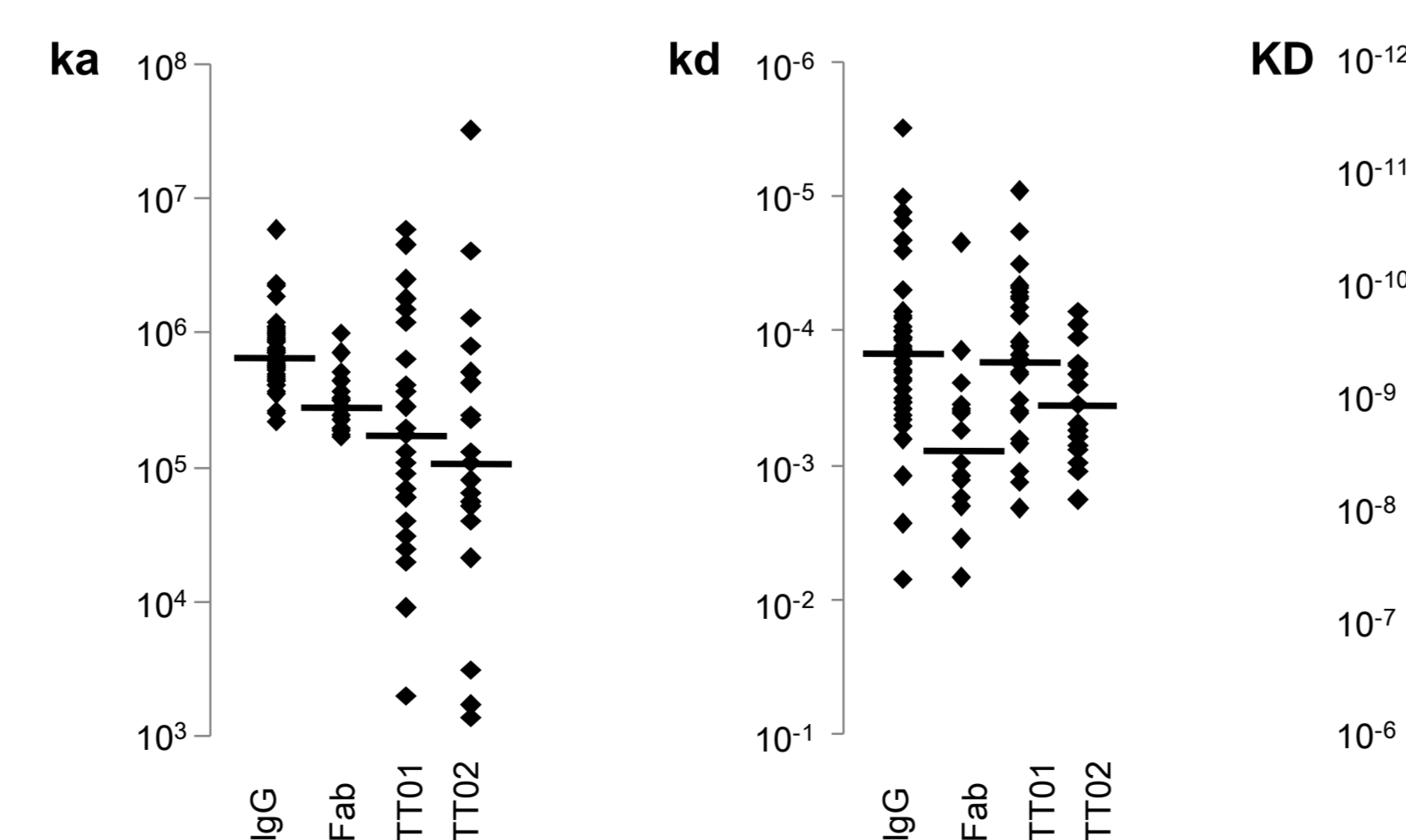
- A single V_L gene in germline configuration was chosen on the basis of its frequent utilization in human immune and naïve repertoires
- Blood was harvested from two tetanus toxoid (TT) vaccinated donors
- V_H genes from all families were harvested and combined with the single V_L gene in a phagemid vector
- Fab phage libraries were selected for binding to tetanus toxoid and the resulting 129 unique clones analyzed
- 53 unique V_H regions (V_H - D_H - J_H recombination events) were identified

Single V_L TT repertoire diversity is very similar to repertoires in which cognate pairing was retained



Black bars, the single V_L TT repertoire; white bars, the combined Symplex TT01/TT02 repertoires^{1,2}; dark grey, rabies virus repertoire³; light grey, West Nile virus repertoire⁴.

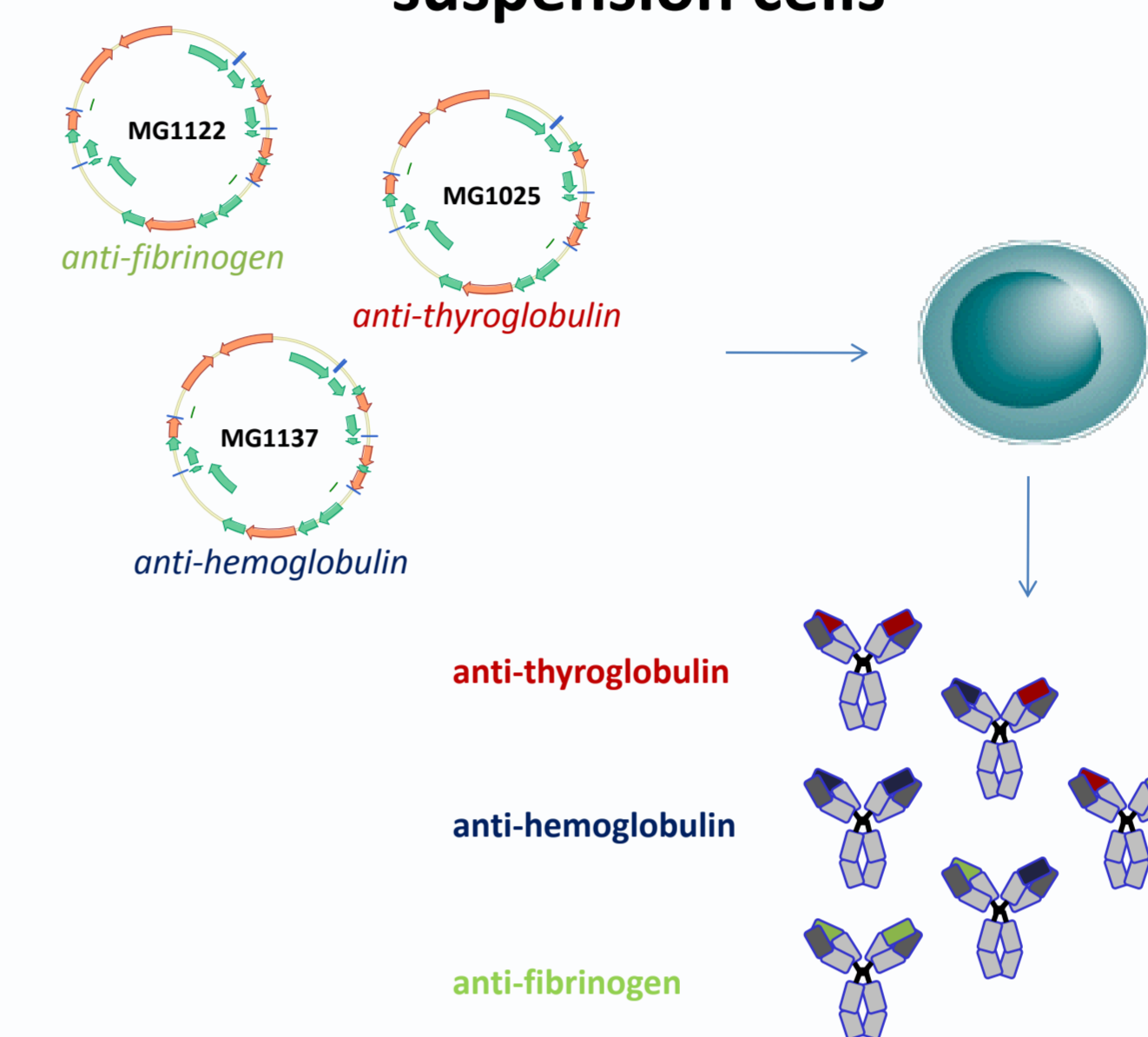
Single V_L TT repertoire affinities are similar to the affinities of TT repertoires in which cognate pairing was retained



On-rates (k_a $M^{-1} s^{-1}$), off-rates (k_d , s^{-1}) and affinities (KD, μM) of TT IgG and Fab fragments. IgG, single V_L TT repertoire IgG; Fab, single V_L TT repertoire Fab; TT01 and TT02, Symplex TT Fab repertoires^{1,2}. The black bars show the median values of each data set.

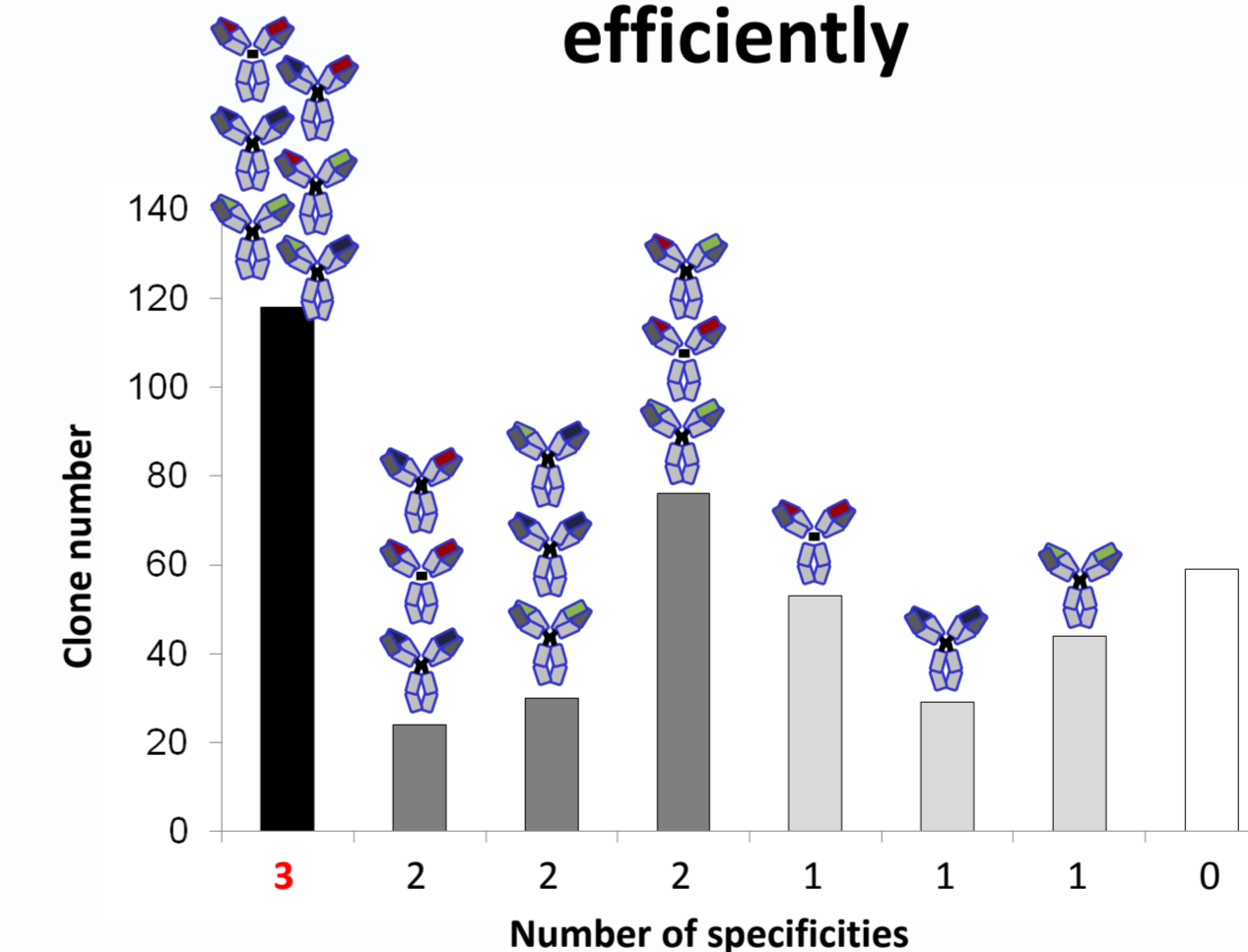
Oligoconics™ Production

Straightforward clone generation in suspension cells



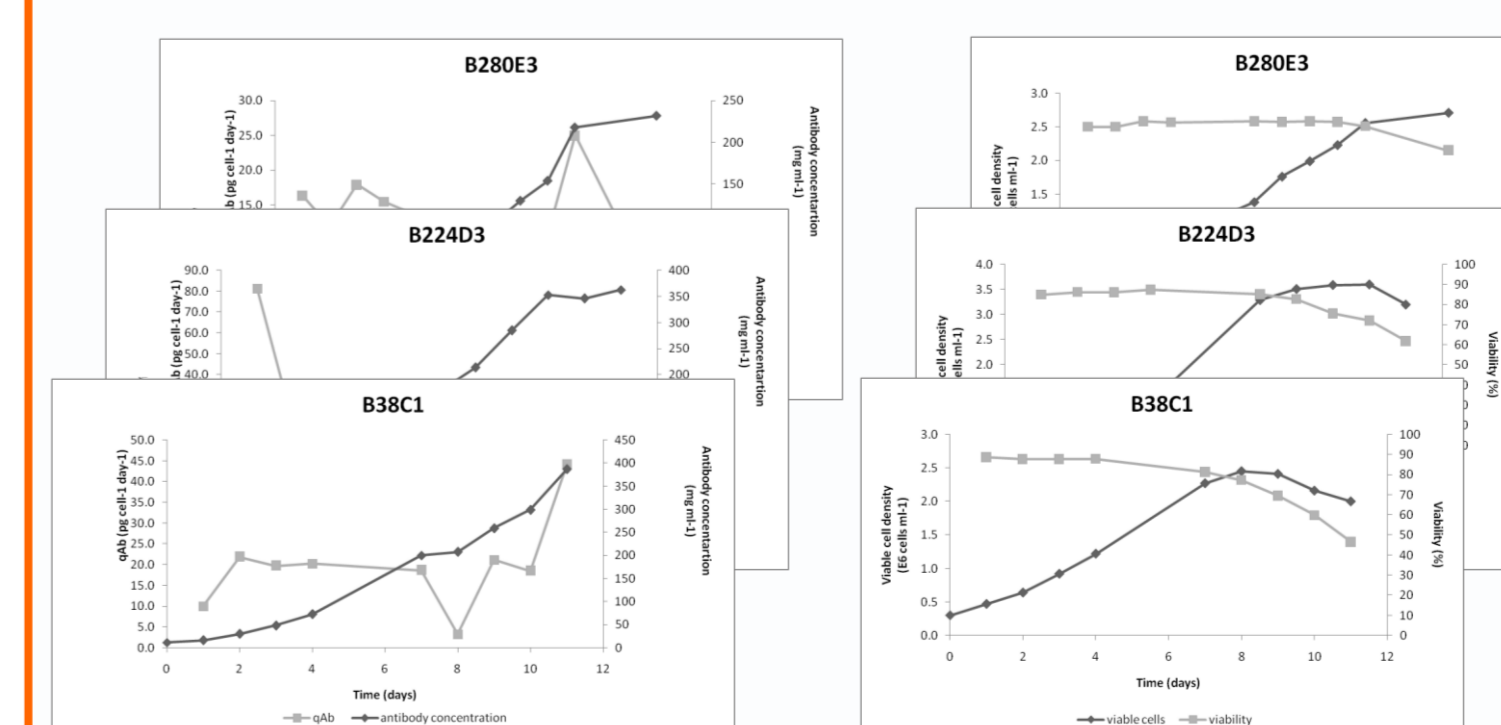
Three plasmids each encoding one heavy chain conferring specificity, an identical light chain and neomycin resistance were transfected into suspension Per.C6® cells. Clones positive for all three specificities were subcloned by limiting dilution and growth- and production characteristics of these clones studied.

Oligoconics™ clones are generated efficiently



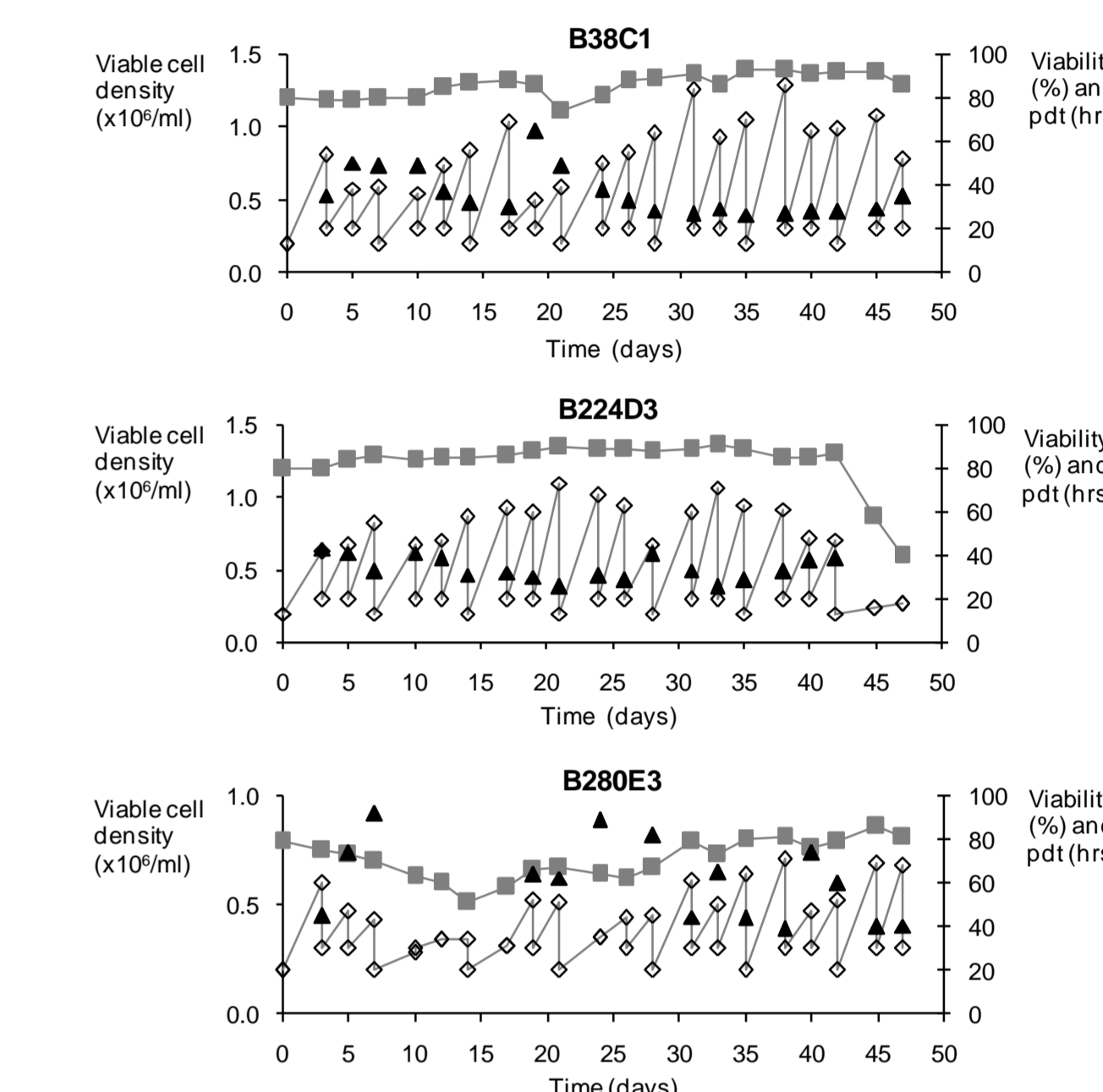
27% of the clones express all three specificities; the other clones express two, one or no specificities.

Oligoconics™ clones have normal production characteristics



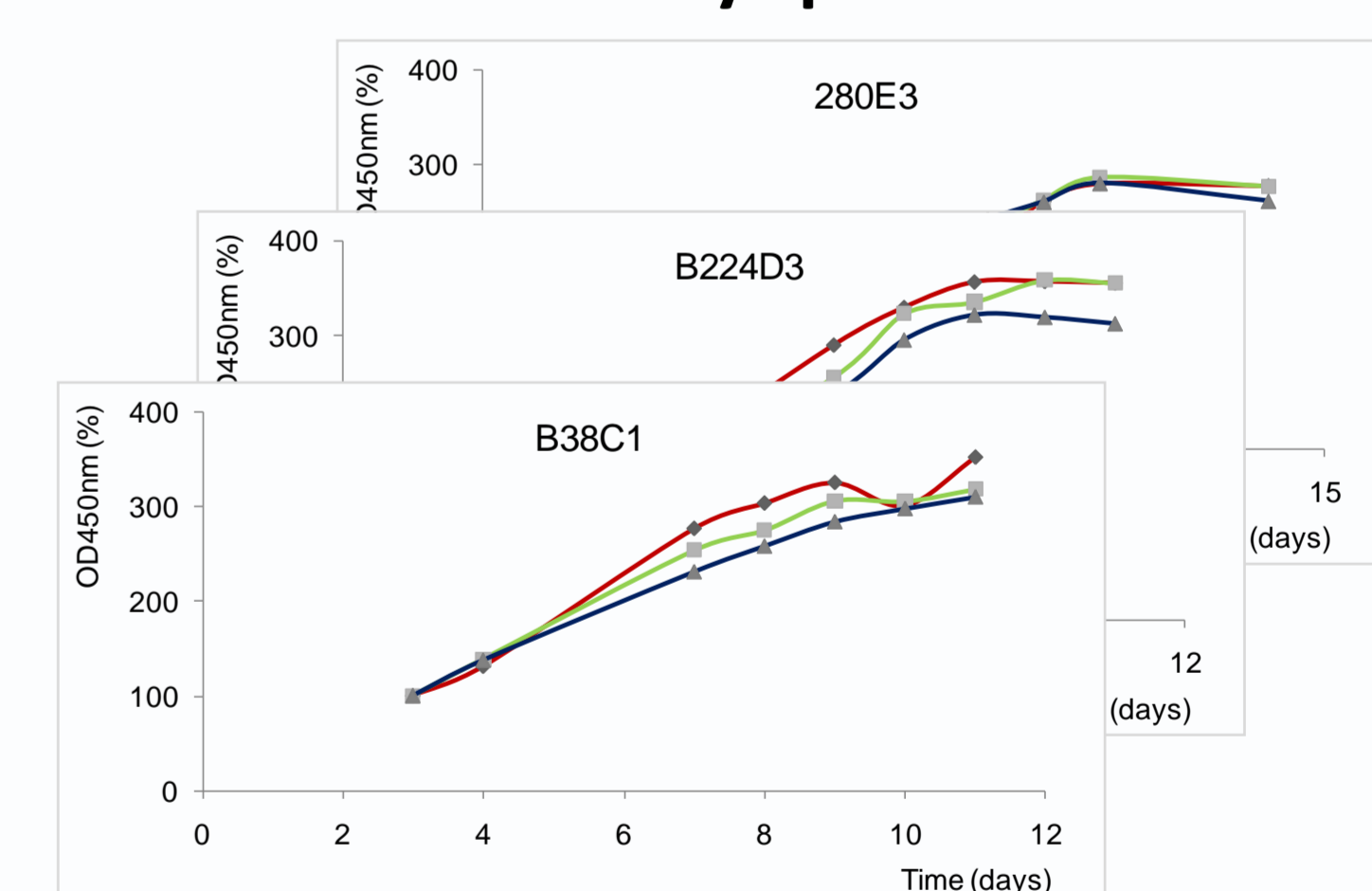
Non-fed batch production runs. Cells were inoculated at a concentration of 0.3×10^6 cells/ml in VPRO medium in a shaking flask. At the days indicated, specific production (qAb), total antibody concentration, viable cell density and viability were measured.

Oligoconics™ clones have normal growth characteristics



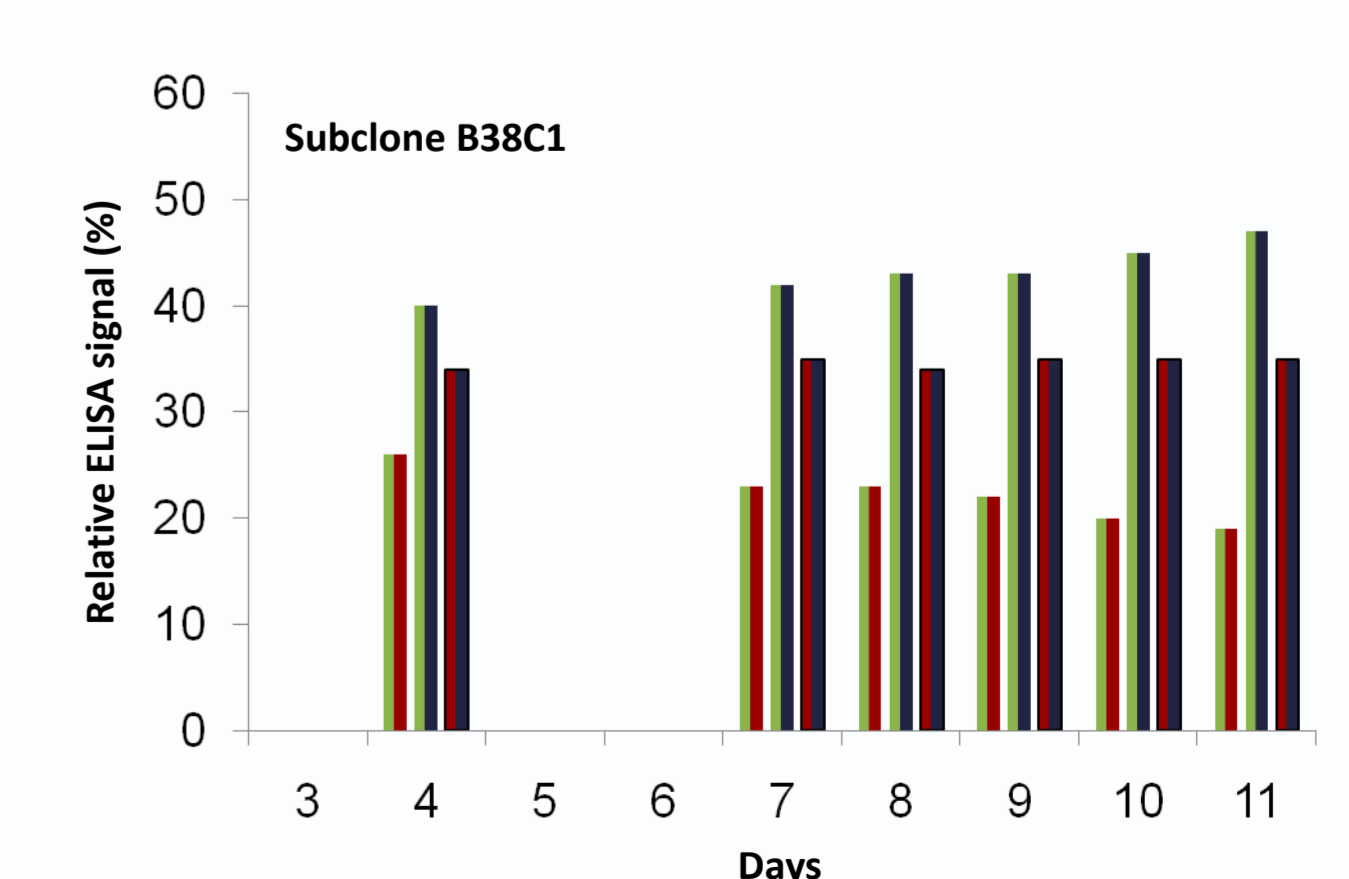
Oligoconics™ clones have a viability and passage doubling time in line with single-IgG producing Per.C6® clones. (cell density (\diamond), viability (\square), and passage doubling time (pdt, \blacktriangle))

Oligoconics™ clones stably express three antibody specificities



Protein expression of the 3 specificities is linear in batch cultures up to 14 days. Anti-fibrinogen / thyroglobulin / hemoglobin.

Oligoconics™ clones express bispecific antibodies in constant ratios



Bispecific protein levels were measured by double antigen capture ELISA during batch production runs and found to be stable. Anti-fibrinogen / thyroglobulin / hemoglobin.