Supplementary Table 1: List of oligonucleotides used for NGS sample preparation

L3-Fwd: CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCATCCGCCCGGAAGACTTCGCAACTTA

L3-Rev: CCTCTCTATGGGCAGTCGGTGATATCTCCACCTTGGTACCCTG

H1-Fwd: CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCATCCGCCGTTTGTCCTGTGCAGCTTC

H1-Rev: CCTCTCTATGGGCAGTCGGTGATCCCTTACCCGGGGCCTGACG

H2-Fwd: CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCATCCGCCCCCGGGTAAGGGCCTGGAA

H2-Rev: CCTCTCTATGGGCAGTCGGTGATCTTATAGTGAAACGGCCCTTGACGCT

H3-Fwd: CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCATCCGCAGGACACTGCCGTCTATTAT

H3-Rev: CCTCTCTATGGGCAGTCGGTGATACGGTGACTAGTGTACCTTG

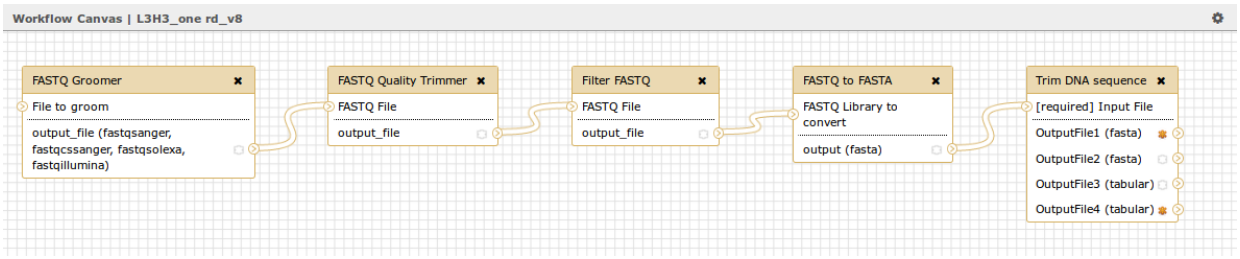
L3-H3 Seq: ACGTTCGGACAGGGTTATTATTGTGCTCGC

L3-H1 Seq: ACGTTCGGACAGGGTGCTTCTGGCTTCAAC

H1-H2 Seq: CACTGGGTGCGTCAGCTGGAATGGGTTGCA

H2-H3 Seq: TATGCCGATAGCGTCTATTATTGTGCTCGC

Oligonucleotides used for NGS amplicon preparation contained the following features: forward primers contained adaptor (blue), key (green), barcode (red) and antibody framework (black) regions, and reverse primers contained truncated P1 (orange) and antibody framework (black) regions. Adaptor and truncated P1 sequences were included in the primers for facilitating emulsion PCR following amplicon preparation. To amplify a CDR of interest from phage pools, for example CDRH3, H3-Fwd and H3-Rev primers were used in the PCR reaction. To generate the L3-H3 CDR strip from phage pools, one oligonucleotide (L3-H3 Seq) was used in the Kunkel mutagenesis reaction. To generate the L3-H1-H2-H3 CDR strip from phage pools, three oligonucleotides (L3-H1 Seq, H1-H2 Seq and H2-H3 Seq) were used in the Kunkel mutagenesis reaction. To amplify L3-H3 or L3-H1-H2-H3 CDR strips, L3-Fwd and H3-Rev primers were used in the PCR reaction.



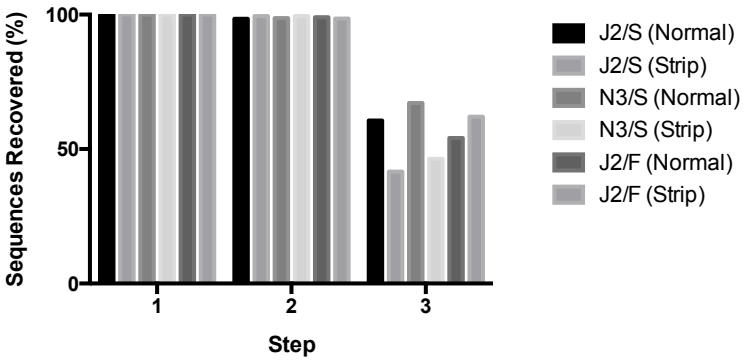
Supplementary Figure 1: NGS Galaxy Workflow.

1.) **FASTQ Groomer:** Converts Torrent Server outputted FASTQ file, into the sanger FASTQ format (fastqsanger) to allow analysis with the downstream tool, FASTQ Quality Trimmer. (Blankenberg D, Gordon A, Von Kuster G, Coraor N, Taylor J, Nekrutenko A; Galaxy Team. Manipulation of FASTQ data with Galaxy. Bioinformatics. 2010 Jul 15;26(14):1783-5.)
2.) **FASTQ Quality Trimmer:** Trims from 3' end with a window size of 1 and step size of 1. Trimming continues until the base has a quality score of 17 or greater. There is no minimum or maximum number of bases to trim (Blankenberg D, Gordon A, Von Kuster G, Coraor N, Taylor J, Nekrutenko A; Galaxy Team. Manipulation of FASTQ data with Galaxy. Bioinformatics. 2010 Jul 15;26(14):1783-5).
3.) **Filter FASTQ:** Filters out entire sequences that are smaller than the smallest possible CDR (27bp) or have a combined quality score less than 17.0 (Blankenberg D, Gordon A, Von Kuster G, Coraor N, Taylor J, Nekrutenko A; Galaxy Team. Manipulation of FASTQ data with Galaxy. Bioinformatics. 2010 Jul 15;26(14):1783-5).
4.) **FASTQ to FASTA:** removes the quality information from the file to allow analysis with downstream tools (FASTX-toolkit by Assaf Gordon).
5.) **Trim DNA Sequence**: R script which has been ported to work within galaxy. Searches for and trims the sequences that immediately precede and follow the CDR(s) in the CDR strip of interest. This tool also identifies the template that was used for library creation and counts its occurrences and extracts the CDR(s). Further, the tool counts all unique CDRs and determines their relative frequency. This is outputted into a tabular file. Further, this tool also outputs a fasta file containing all trimmed sequences for use in other downstream tools if desired.

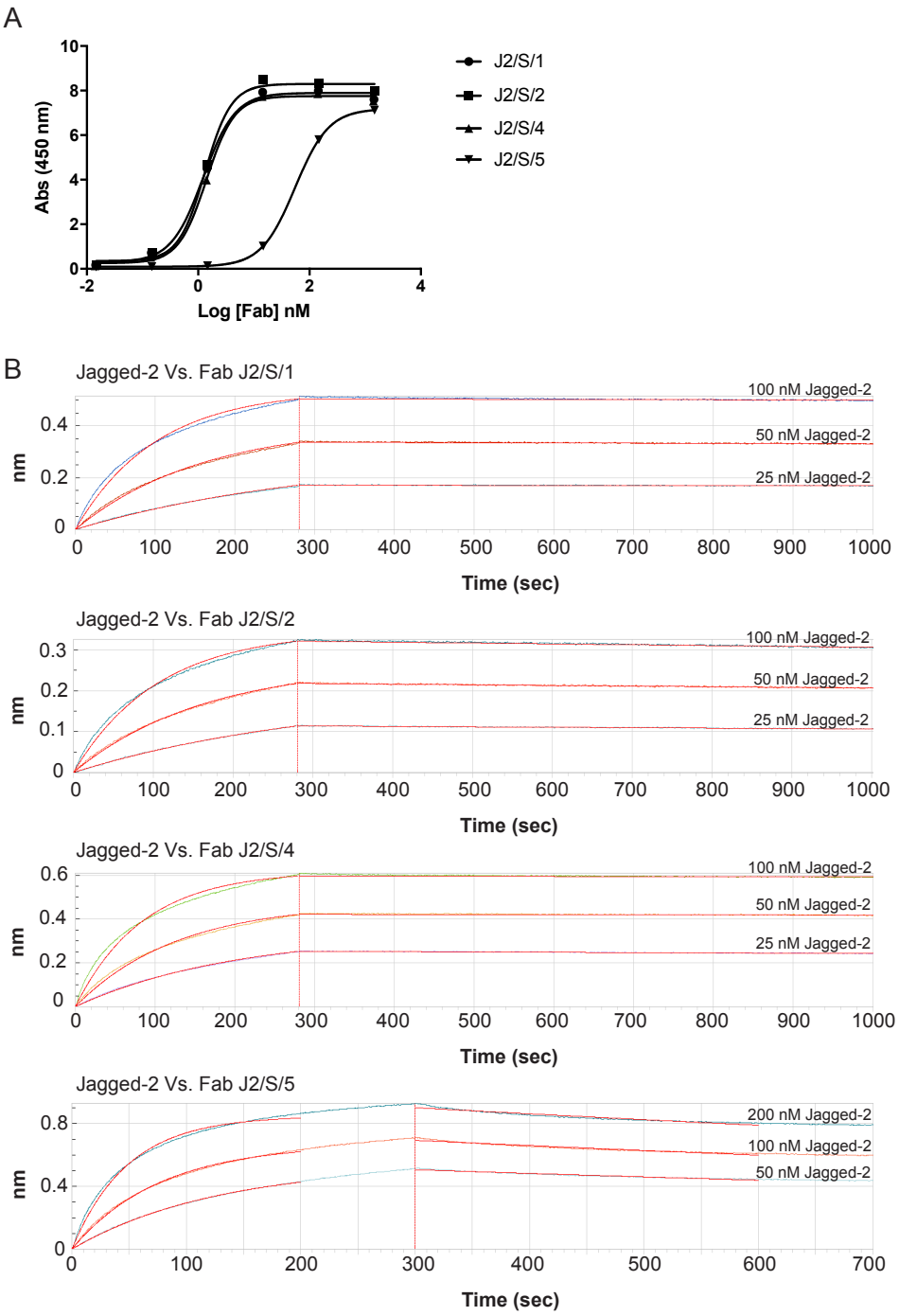
Galaxy References:

1. Goecks, J, Nekrutenko, A, Taylor, J and The Galaxy Team. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol.* 2010 Aug 25;11(8):R86.
2. Blankenberg D, Von Kuster G, Coraor N, Ananda G, Lazarus R, Mangan M, Nekrutenko A, Taylor J. "Galaxy: a web-based genome analysis tool for

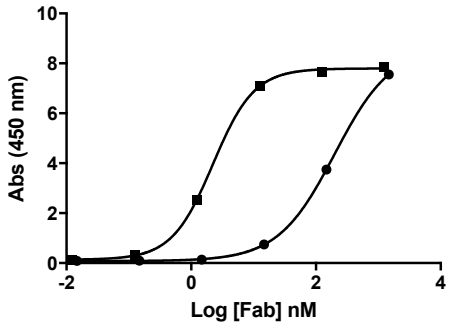
experimentalists". *Current Protocols in Molecular Biology*. 2010 Jan; Chapter 19:Unit 19.10.1-21.
3. Giardine B, Riemer C, Hardison RC, Burhans R, Elnitski L, Shah P, Zhang Y, Blankenberg D, Albert I, Taylor J, Miller W, Kent WJ, Nekrutenko A. "Galaxy: a platform for interactive large-scale genome analysis." *Genome Research*. 2005 Oct; 15(10):1451-5.
4. Daniel Blankenberg, Gregory Von Kuster, Emil Bouvier, Dannon Baker, Enis Afgan, Nicholas Stoler, the Galaxy Team, James Taylor and Anton Nekrutenko, "Dissemination of scientific software with Galaxy ToolShed," in *Genome Biology* 2014, 15:403, doi:10.1186/gb4161



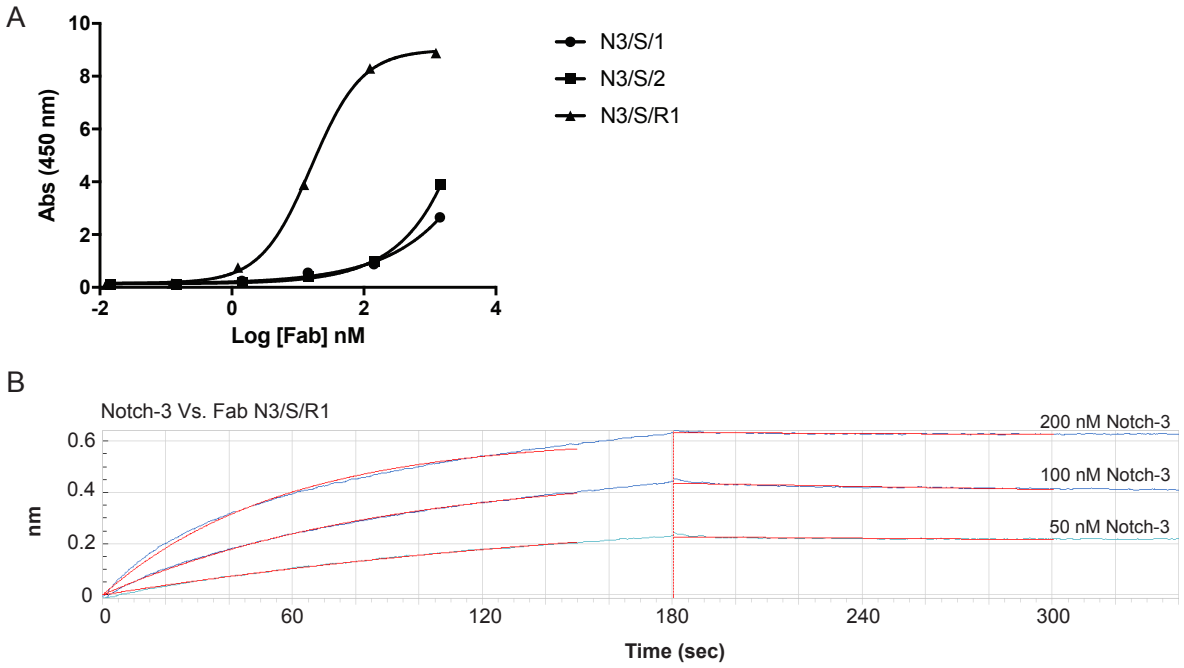
Supplementary Figure 2: Sequences recovered by single CDR NGS method vs CDR strip generation NGS method. NGS processing steps: (1) Percentage of reads after pre-processing steps. (2) Percentage of sequences that contain 5' region of interest. (3) Percentage of sequences that contain CDR(s) of interest.



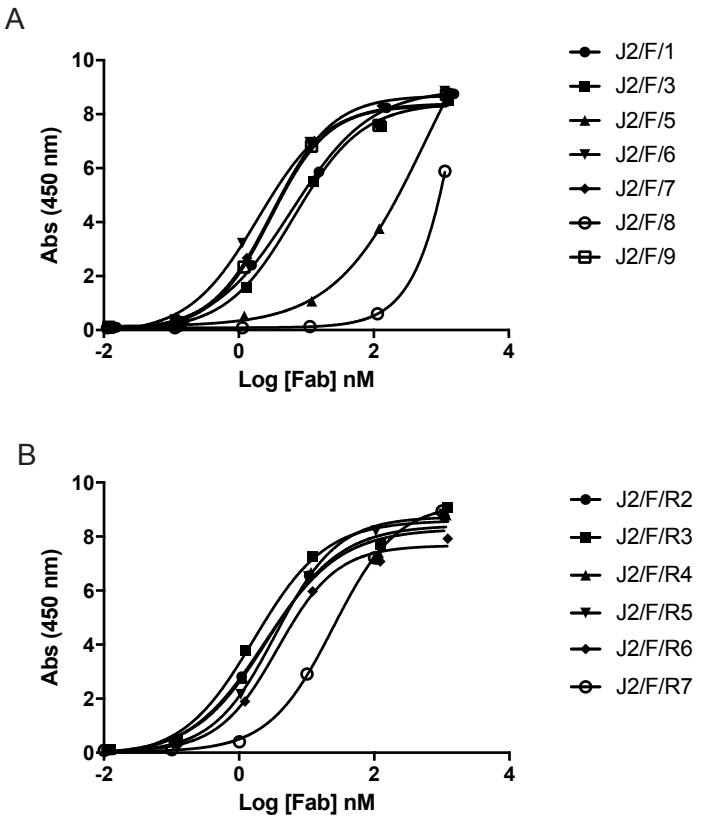
Supplementary Figure 3: Binding analyses for Jagged-2 Fabs isolated from Library-S. **(A)** Analysis of Jagged-2 Fabs J2/S/1, J2/S/2, J2/S/4, and J2/S/5 binding to immobilized Jagged-2 by Fab-ELISA. EC_{50} values were calculated by fitting the data to the one-site specific-binding equation. **(B)** Analysis of Jagged-2 binding to sensor-immobilized Fabs J2/S/1, J2/S/2, J2/S/4, and J2/S/5 by bio-layer interferometry. K_D values were obtained by fitting the association and dissociation data points to a 1:1 binding model. EC_{50} and K_D values are shown in Figure 3B.



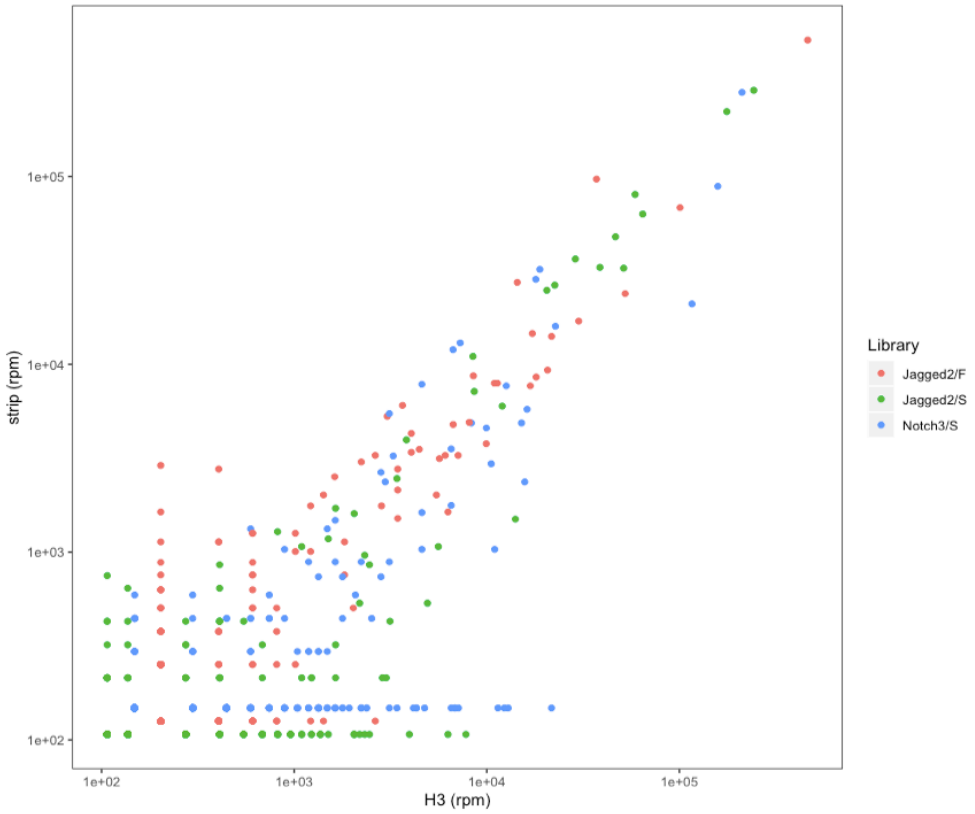
Supplementary Figure 4: Analysis of Jagged-2 rare Fabs J2/S/R1 (solid circles) and J2/S/R2 (solid squares) binding to immobilized Jagged-2 by Fab-ELISA. EC_{50} values were calculated by fitting the data to the one-site specific-binding equation. EC_{50} values are shown in Figure 4E.



Supplementary Figure 5: Binding analyses for Notch-3 top and rare Fab clones isolated from Library-S. **(A)** Analysis of Notch-3 Fabs N3/S/1, N3/S/2 and N3/S/R1 binding to immobilized Notch-3 by Fab-ELISA. EC_{50} values were calculated by fitting the data to the one-site specific-binding equation and are shown in Figure 5C. **(B)** Analysis of Notch-3 binding to sensor-immobilized Fab N3/S/R1 by bio-layer interferometry. K_D was obtained by fitting the association and dissociation data points to a 1:1 binding model. The K_D value was calculated to be 2.65 ± 0.27 nM.



Supplementary Figure 6: Binding analysis for Jagged-2 Fabs isolated from Library-F. **(A)** Analysis of Jagged-2 top Fabs (J2/F/1, J2/F/3, J2/F/5, J2/F/6, J2/F/7, J2/F/8, and J2/F/9) binding to immobilized Jagged-2 by Fab-ELISA. **(B)** Analysis of Jagged-2 rare Fabs (J2/F/R2, J2/F/R3, J2/F/R4, J2/F/R5, J2/F/R6, and J2/F/R7) binding to immobilized Jagged-2 by Fab-ELISA. EC_{50} values were calculated by fitting the data to the one-site specific-binding equation. EC_{50} values are shown in Figure 6C.



Supplementary Figure 7: Correlation between CDRH3 sequences (H3) in NGS data from pre- and post- CDR strip generation. Jagged2/F, Jagged2/S, and Notch3/S had Pearson correlations of 0.9175, 0.9102, and 0.9106, respectively. rpm = reads per million.