

**Supplementary information for**

## **Cell-free expression of functional receptor tyrosine kinases**

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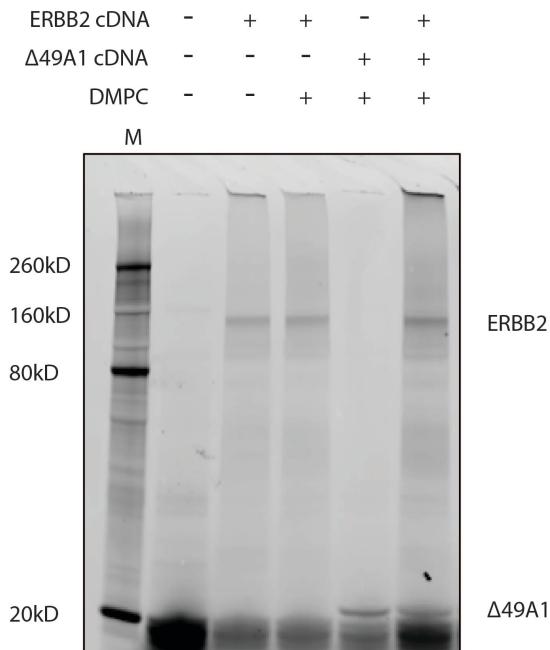
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## Supplementary Figures

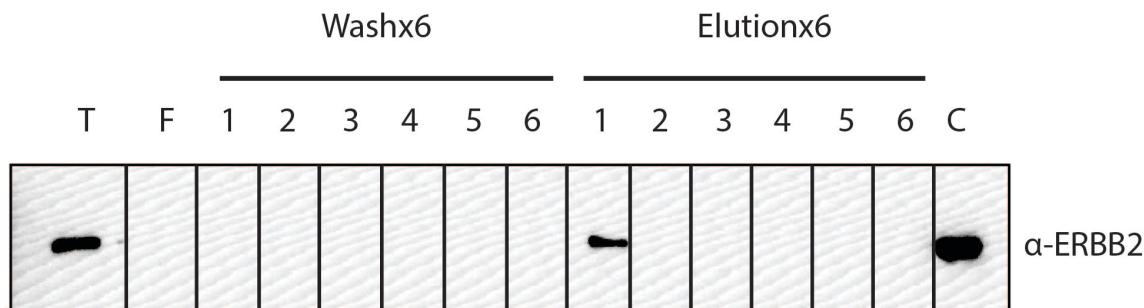
### Supplementary Figure 1



Fluorescent image of denaturing SDS PAGE of cell-free expressed Δ49A1 and ERBB2.

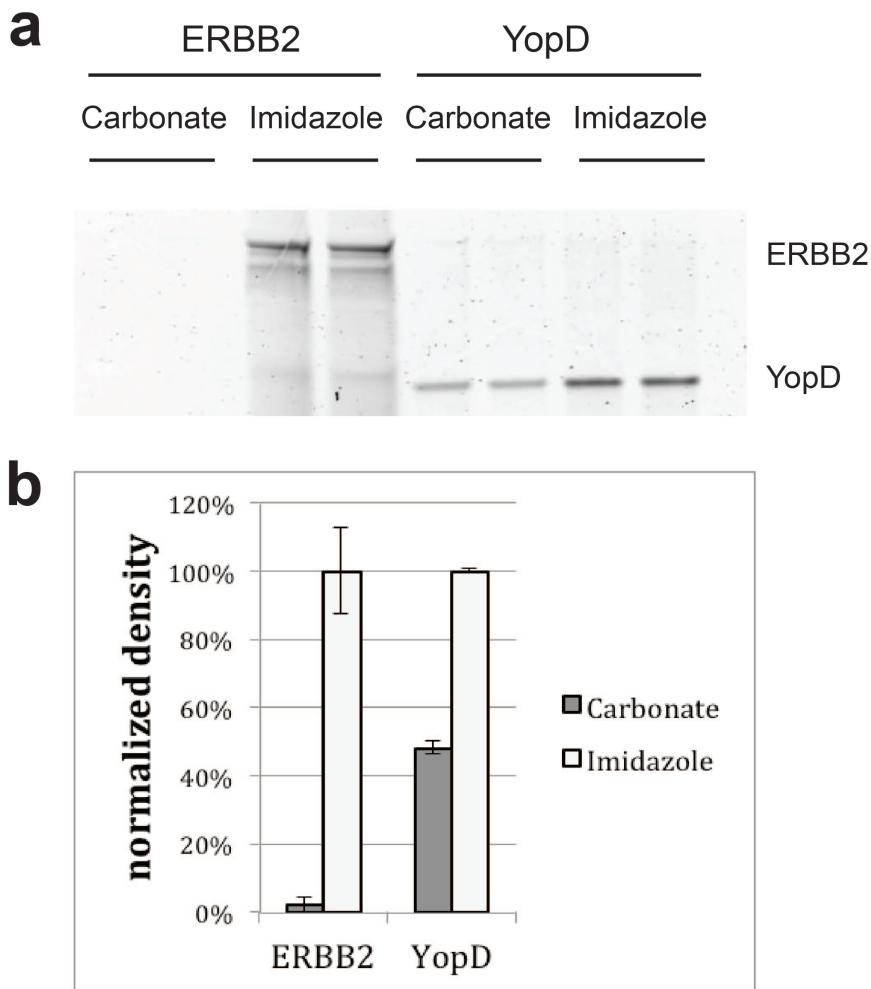
Cell-free expressions were set up using the Expressway Maxi Cell-Free *E. coli* Expression System from Life Technologies according to manufacturer's user manual. Reactions were set up with: no DNA, DNA encoding ERBB2 only, DMPC and DNA encoding ERBB2, DMPC and DNA encoding Δ49A1 (empty NLPs), DMPC and DNA encoding both ERBB2 and Δ49A1 (ERBB2-NLPs). FluoroTect™ GreenLys (Promega) was added for visualizing synthesized protein. Reactions were ended after 18 hours by adding LDS sample buffer from Life Technologies. All samples were boiled for 5mins and resolved by 4-12% SDS-PAGE along with a molecular weight standard (M). Gel images were taken using Molecular Dynamics Typhoon 9410 Molecular Imager from GE Healthcare.

## Supplementary Figure 2



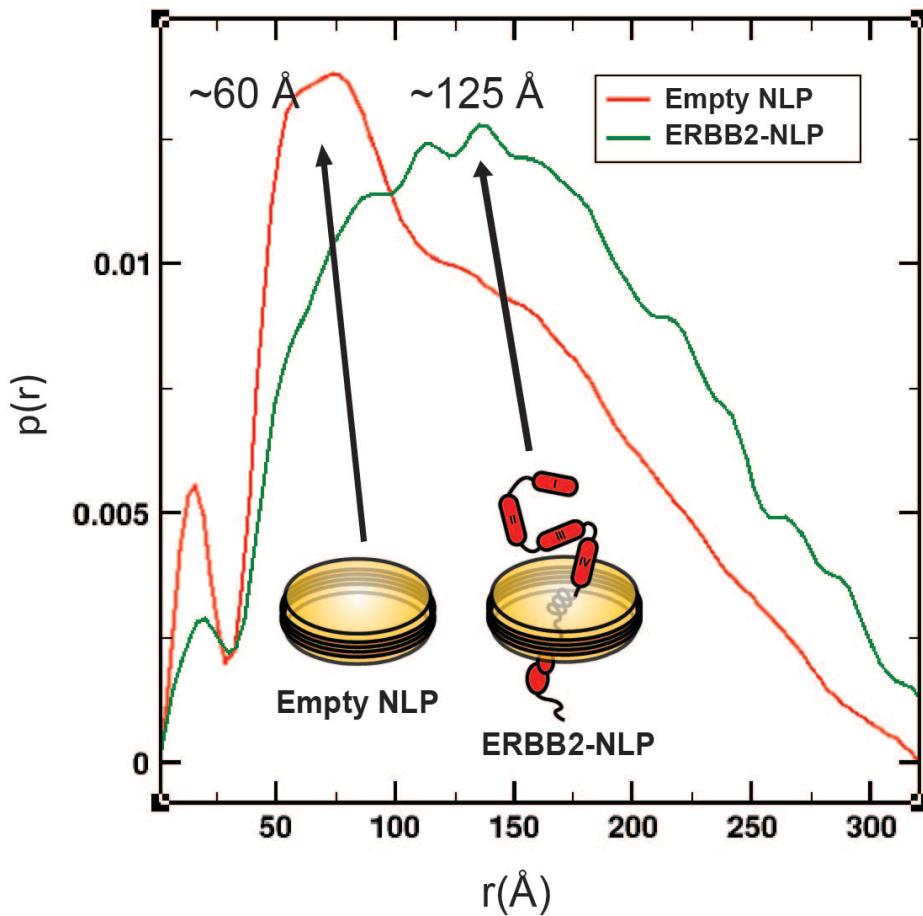
Western blot shows ERBB2-NLP can be Ni purified. Set up 1mL cell-free expression reaction to produce ERBB2-NLP as described in Methods. After 18 hours reaction, a small aliquot of crude was saved as total (T). The rest of the crude mixture was collected and incubated with 0.5 mL Ni-NTA SuperFlow resin (Qiagen) at 4 degree for 2 hours; the unbound crude was collected as flow through (F). Ni resin was then washed with 6mL wash buffer containing increasing concentration (10mM, 20mM, 50mM, two of each, 6 mL total) of imidazole (Wash, 1mLx6). The bound protein was then eluted with 1mL elution buffer containing 400mM imidazole for 6 times (Elution, 1mLx6). Elutions were combined and dialyzed against 1L of TBS, twice; then concentrated with Vivaspin column MWCO=100kDa (C). Final volume is 300uL. Samples were then resolved by SDS-PAGE and western blotting with anti-ERBB2 antibody Ab-3.

**Supplementary Figure 3**



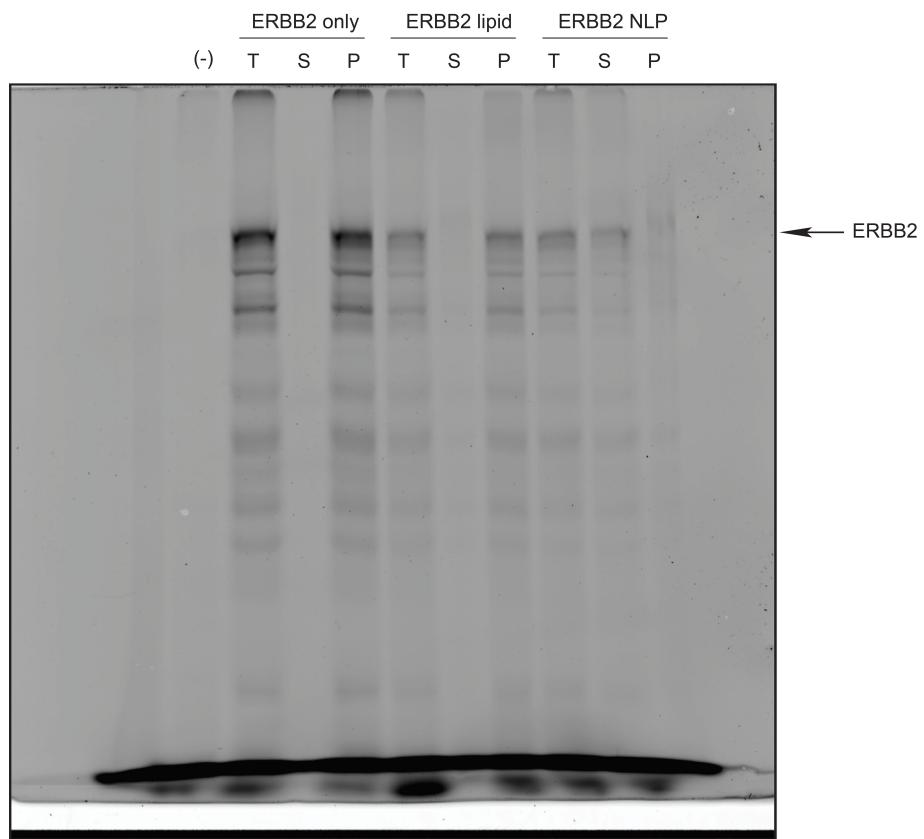
Cell-free expressed ERBB2-NLP and YopD-NLP (Bodipy labeled) were bound to Ni beads through a 6xHis tag located on the scaffold protein Δ49A1. The Ni beads were washed with buffer containing 20mM imidazole, and then eluted with 100 mM sodium carbonate, or 400mM imidazole. The eluents were concentrated and resolved by SDS-PAGE. Pictures of the gel were taken with GE Typhoon 9410 fluorescent imager Ext/Emt: 488 nm/520 nm (a). 48% of YopD protein can be carbonate extracted, whereas only 3% of ERBB2 protein can be carbonate extracted (b).

**Supplementary Figure 4**



Small angle X-ray scattering (SAXS) data for ERBB2-NLPs that were prepared by cell-free expression comparing with empty NLP. NLP and ERBB2 NLP samples were cell-free produced and Ni purified as described. Each sample consisted of 20 microliters of 0.23 mg/ml of total protein. There was a clear difference in the spectra of the NLP with ERBB2 incorporation indicating a 2-fold or more increase in thickness of the nanoparticles compared to the empty NLPs.

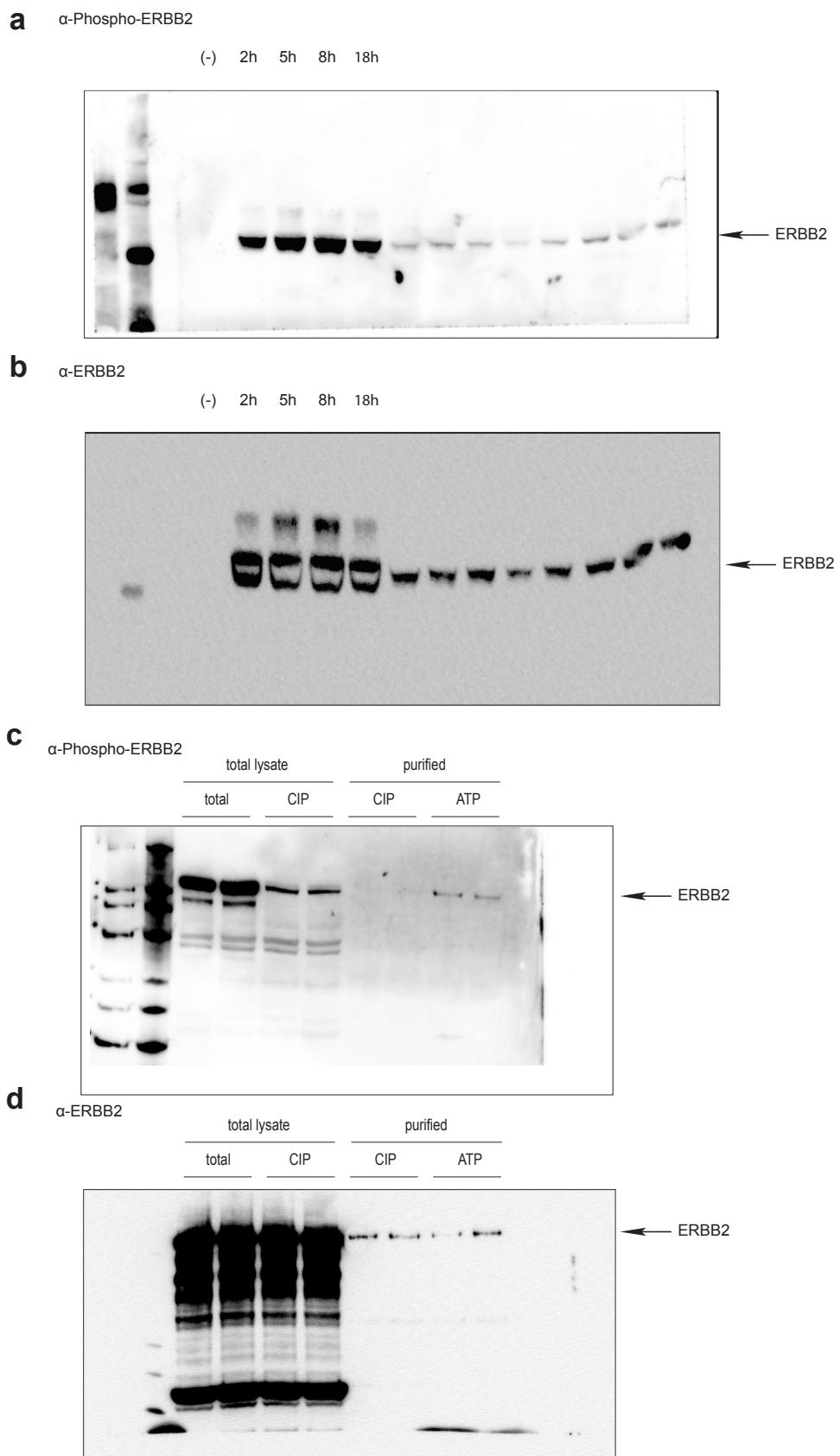
### Supplementary Figure 5

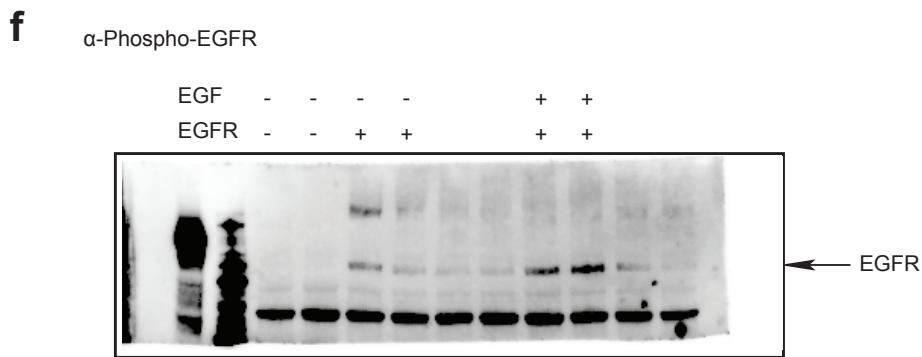
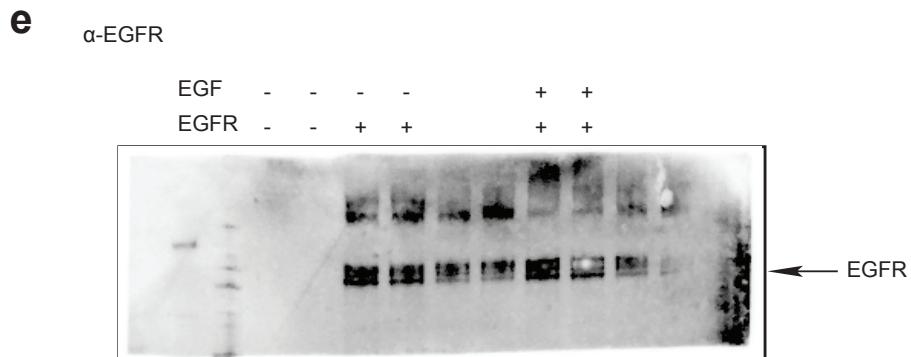


*Full-length versions of gel image presented in figure 1*

ERBB2 was cell-free produced in the presence and absence of DMPC or with DMPC and co-expressed Δ49A1. FluoroTect™ GreenLys (Promega) was added for visualizing newly synthesized ERBB2 protein. After 4 hours of expression, cell-free reactions were centrifuged at 14,000 rpm for 10 minutes. Small aliquots of sample before centrifuging (total, T), the supernatant (soluble, S) and pellet (P) after centrifuging were collected. All samples were loaded along with a cell-free reaction mixture only (-). Gel images were taken using Molecular Dynamics Typhoon 9410 Molecular Imager from GE Healthcare.

## Supplementary Figure 6





*Full-length versions of western blots presented in figure 2*

NLP associated ERBB2 is tyrosine phosphorylated. Cell-free expressions were set up with and without (-) ERBB2 plasmid. Samples were collected at 2hr, 5hr, 8hr and overnight 18hr and resolved by SDS-PAGE and western blotting with anti-phospho-tyrosine ERBB2 antibody pY1248 (a) and anti-ERBB2 antibody Ab-3 (b) after stripping.

The NLP associated ERBB2 is phosphorylated independent of protein expression. Cell free expressed ERBB2 is treated with calf-intestinal alkaline phosphatase (CIP) and Ni purified. The purified ERBB2-NLPs are then incubated with ATP, Mn<sup>2+</sup>, Mg<sup>2+</sup> and buffer to allow for re-phosphorylation. Samples are resolved by SDS\_PAGE and western blotting with anti-phospho-tyrosine antibody 4G10 (c) and anti-ERBB2 antibody Ab-3 (d).

NLP associated EGFR is also phosphorylated. Presence of EGF in the cell free reaction increases the level of phosphorylation. EGFR-NLPs showed low level of phosphorylation during cell-free expression. Adding EGF, the natural ligand of EGFR, increases the phosphorylation. Cell-free expressions were set up with and without (-) EGFR plasmid, with and without EGF. After 8hrs reaction, cell-free mixtures were resolved by SDS-PAGE and western blotting with anti-EGFR (e) comparing to western blotting with anti-phospho-tyrosine EGFR antibody pY1110 (f).

**Supplementary Figure 7.**

Alignment of the DNA sequences of human ERBB2 and EGFR genes with the DNA sequences that were codon optimized for *E.coli* expression. A dark block represents where the two sequences share the same base.

DNA encoding human ERBB2: comparing E.coli codon optimized sequence to human gene sequence.

E.coli	ATGGAAATTGGCTGCAATTGTGTCGGTTGGGGTTTGTTGGTGGCATGGCTGCCCGCCCTGGAGGGGGCTCCACCCAAAGTGTGTACCGGCAC	GACATGAAGTTGC	301
Human	ATGGAGCTGGCGGCCCTTGCGCTGGGGCTCCTCTCGCCCTCTGCCGAGCACCCAAAGTGTGCACCCGCAC	GACATGAAGCTGC	301
E.coli	GGCTCCCTGGTTCCCCAGAAACCACCTCGAATATGTTGGGCCATCTCTATCAAGGGTGCCTGGGGTAATCTGGAACCTGACCTGCCTAC	GGCTCCCTGGCTAC	301
Human	GGCTCCCTGGCAGTCCCAGAACCCACCTCGACATGCTCGCCACCTCTACCAAGGGCTGGGGTGCAAGGGAAACCTGGAACCTACCTGCCTAC	GGCTCCCTGGCTAC	301
E.coli	GAACGGTCCCTGAGCTTCTGCAAGATACTCCAAAGAAGTGCAGGGCTACGTCTGATTGCCCCACAAACAGGTCGCCAGGTCCCCTGTCAGCGGCTGGC	301	301
Human	CAATGCCACCCCTGCTCTCTGAGGATACTCCAGGGTGCAGGGCTACGTCTGATCGCTCACACCAAGTGAAGGCAAGGTCCCCTGTCAGCGGCTGGC	301	301
E.coli	ATTGTTCGGAAACCCAGCTCTCGAGGAIAACTACGCTTGGCGCTGCTGGATAATGTTGACCCCTGAAACAAATACCAACGCCGGTAC	TGGTGCCTCCC	301
Human	ATTGTTCGGAGGACCCAGCTCTTGAGGAAACATATGCTCTGGCCCTGCTGGATAACATGGACAGACCCCTGAAACAAATACCAACGCCGGTAC	TGGTGCCTCCC	301
E.coli	GGGGAGGACTCCCGGAACTGCGAGCTCGGGAGCCCTGACCGAAATTCTGAAAGGAGGGCTGCTGATTCACAGAACCCCTGTCAGTACGGGACACTAT	301	301
Human	GAAGGAGGCTCGGGAGCTGCGAGCTCGAAGCCCTCACAGACATCTGAAAGGAGGGCTTGTATCGAGGGAAACCCCTGTCAGTACGGGACACTAT	301	301
E.coli	CTTGTGGAAAGGACATCTTCAACAAGAACACCAGCTCGCGCTGACCTGTATGACACCCATTCGAGCTCTCACACTGATAGACACCCACCGCTCTCGGATGTAAAG	601	601
Human	CTTGTGGAAAGGACATCTTCAACAAGAACACCAGCTCGCGCTGACCTGTATGACACCCATTCGAGCTCTCACACTGATAGACACCCACCGCTCTCGGATGTAAAG	601	601
E.coli	GGCTCCCGCTGTTGGGGTGAATCCAGCGAGGATTTGCGCTGCTGGCTCCAGGCTGCGACGGCGCTGCGTGCAGGTCGCCCCCTGCAAGGGCCACTGCGC	301	301
Human	GGCTCCCGCTGCTGGGGAGAGAGTTGAGCTGAGGCTGAGGCTGCGACGGCTGCGTGCAGGTCGCCCCCTGCAAGGGCCACTGCGC	301	301
E.coli	ACTCTTGGCCATGAGCAGTGCAGGGTTGCAAGCTCTGACTGCTGGCTCCAGGACTCTGACTGCTGGCTCCAGGACTCTGACTGCTGGCTCCAGGACTCTGAGCTGCA	301	301
Human	ACTCTTGGCCATGAGCAGTGCAGGGTTGCAAGCTCTGACTGCTGGCTCCAGGACTCTGACTGCTGGCTCCAGGACTCTGACTGCTGGCTCCAGGACTCTGAGCTGCA	301	301
E.coli	CTGCCCTGGCTCGTACGTAACACAGACACATTTCGAGGACATGGAAATCCGGAAGGCTGATAGCTTGGAGCGCTTGTGCGTGAAGGGATGTCGCTGCTGCTGCTG	901	901
Human	CTGCCCTGGCTCGTACGTAACACAGACACATTTCGAGGACATGGAAATCCGGAAGGCTGATAGCTTGGAGCGCTTGTGCGTGAAGGGATGTCGCTGCTGCTG	901	901
E.coli	TACAACCTACCTCTCACCGATGGCGAGCTGAGCTGACGGCTGCTGCCCCCTGCACTAACCAAGGAAGTTACTGCGGAGGACGGCACCCAGCCCTGCGAGAAAT	3001	3001
Human	TACAACCTACCTCTCACCGATGGCGAGCTGAGCTGACGGCTGCTGCCCCCTGCACTAACCAAGGAAGGTTACTGCGGAGGAGGATGGAACACAGCCCTGCGAGAAAT	3001	3001
E.coli	TTTCTAAACCATGTGCTCGCGTGCTGTAAGCTTGGGATGGAACATCTGCGGGAGTGTGCTGTTGGCTATGGCTCTGGGATGGAGCACTTGGCGAGGAGTACCGAGTACCTGCTGGCTG	1101	1101
Human	TCAGCAAGGGCTGTGCGGGAGTGTGCTGTTGGCTATGGCTCTGGGATGGAGCACTTGGCGAGGAGTACCTGCTGGCGAGGAGTACCGAGTACCTGCTGGCTG	1101	1101
E.coli	GAAAGCTTGGAAAGAGATACTGGTACCTCTACATCTCCGCGCTGGCGAGACGGCTGCCTGATCTGAGCGTGTTCAGAAAGTCTGCAAGTATTCGAGCTGCA	3001	3001
Human	GAGAGCTTGGAAAGAGATCACAGGTTACCTCTACATCTCCGCGCTGGCGAGACGGCTGCCTGACGAGCTGCAAGTATTCGAGCTGCA	3001	3001
E.coli	GCATCTTCCCAAATGGCGCTATAGCTGAGCTGCGAGGGCTGGGCAATTCTGCGCTGGGCCCTGCGACGGCTGGGCACTGAGCTGGGACTCTGCGACT	1401	1401
Human	GAATCTTCCCAAATGGCGCTATAGCTGAGCTGCGAGGGCTGGGCAATTCTGCGCTGGGCCCTGCGACGGCTGGGCACTGAGCTGGGACTCTGCGACT	1401	1401
E.coli	GATCCATCACAAACACTCATCGTGTCTTGTACACTGTCCCTGGGAATCACGCTTGGGATCACTGTTGGCCCAATCCGACCAAGGCGTGCCTCCACACCGCTTATCGCCCT	1501	1501
Human	CATCCACATTAACACCCACCTCTGCTCGTACACGGCTGGGCACTGAGCTTGGGCAACCCGACCAAGCTCTGCTCCACACTGCGAACCCGGCA	1501	1501
E.coli	GAGGACGATAGCGTGGTGAGGTCTGGGCTGCGACGGCTGGGCGAGGGCTGGGCACTGGGACTCTGGGCTTCCAGGGCCACCCAGTGTGTCAGGCACTGGCAGGACT	1601	1601
Human	GAGGACGACTGTGCGAGGGCTGGGCTGCGACGGGCTGGGCGAGGGCTGGGCACTGGGACTCTGGGCTTCCAGGGCCACCCAGTGTGTCAGGCACTGGCAGGACT	1601	1601
E.coli	TTCGCCGGGTCAAGGAATGTTGTTGGAAAGAATGTCGCGTGTGCAAGCTTGGCTCGAGTATGTGAAAGGAGCCACTGCTGCAAGGGCTTGGGAGTATGTGAA	1701	1701
Human	TCCCTCCGGCCAGGAGTGTGCGTGGAGGAATGTCGCGAGTACTGCAAGGGCTTGGGAGTATGTGAAAGGAGCCACTGCTGCAAGGGACTTGGGAGTATGTGAA	1701	1701
E.coli	TCAACCAAAACGGTAGCGTTACGTTGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	1801	1801
Human	TCACCGCCAGAAATGGCTAGTGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	1801	1801
E.coli	CGAAGCGGGTCAAGCGGATCTGAGCTATATGCTTATCTGGAAATTCCGGATGAAAGAGGGAGCTTGAGGCTATGCGATCAATGGCACCCACAGCT	1901	1901
Human	CGAAGCGCTGTGAAACCTGACCTCTCCATAGTCCCAGTCTGGAAATTCCGGATGAAAGAGGGAGCTTGAGGCTATGCGATCAATGGCACCCACAGCT	1901	1901
E.coli	GCGTTGACCTCGATGATAAAAGGCTGTCAGCAGGACGGCAGCGCAAGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2001	2001
Human	GTGTTGACCTGGATGAAAGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2001	2001
E.coli	GGCTGTGTGGGGTATCTCTGATCAACCGCCGAAACAAAGATCCCGAAGTACACCATGCGCTCGCTGGCAAGGATGGCTGGGCTGGGCTGGGCTGG	2101	2101
Human	GGGGGGCTGTTGGGGATCTCTGATCAACCGCCGAAAGATCCCGAAGTACACCATGCGCTGGGAGGAGCTGGCTGGGAGGAGCTGGGCTGGGCTGG	2101	2101
E.coli	ACTCCATCTGGCGCGATGCTTAATCAACCGACAGATGCGCATTTTGAAGAGACTGAGCTCCGCAAGTAAAGTGTCTGGGAGCTGGCTGG	2201	2201
Human	AGACCTAGGGGAGCGATGCTCAACACGGCGCAGATGCGATCTCTGAAAGAGACGGAGCTAGGAGGTGAGCTTGGGATCTGGGCTGG	2201	2201
E.coli	TTCACAAAGGTTATTTGGATTCCGGACGGTGAGAACGTTAACATCCCCTGGCAATCAAGTAAAGTGTGCTGGGAGTATGCAACCCACATCCCCTGAGGAAAT	2301	2301
Human	TCTACAAAGGCACTCTGGATCCCTGATGGGAGAATTTGGGAGGAAATGTCGCACTGGGAGTATGCAACCCACATCCCCTGAGGAAAT	2301	2301
E.coli	TCTGGATGAGGGCTATGTGATGGCGGTGTTGGGGAGCCCTTACGTGACGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2401	2401
Human	CTTAGAGGAAAGCATACGTTGATGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2401	2401
E.coli	ATGCCCTACCGGTTGGCTGTGACCTGATGGGAAACCCGCGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2501	2501
Human	ATGCCCTATGGCTGGCTCTTAAAGGATGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2501	2501
E.coli	CCTATCTCGAGATGTCCGGCTGGTCTGCGATCTGGCAAGCCGGATGTTCTGGGAGCTGGTCAAGGGACTTGGCCGCTGGTCAAGAGT	2601	2601
Human	GCTACCTCGAGGATGTGCGGCTGCTGACACAGGGACTTGGCCGCTGGTCAAGGGACTTGGCCGCTGGTCAAGAGTCCAACTGTC	2601	2601
E.coli	GCGCTTGGACATCGACGGACTGATTAATCTGCGACGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2701	2701
Human	TGGCTGCTGGACATCGACGGACTGATTAATCTGCGACGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2701	2701
E.coli	CATCAGACGACCTGGTTCCTATGGCGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2801	2801
Human	CACCAAGCTGATGTTGGAGTATGGCTGTCAGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2801	2801
E.coli	TGCTGGAAAGGGAGAACGGCTGCGTCAAGGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2901	2901
Human	TGCTGGAAAGGGAGAACGGCTGCGTCAAGGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	2901	2901
E.coli	GCGTTTCGGGAATTGGTTAGCGAGTCAGCCGGATGGCGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3001	3001
Human	AAGATTCGGGAGTTGGTGTCTGAATTCTCCGGCATGGGCAAGGGACCCGAGCGCTTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3001	3001
E.coli	GATAGCACTTTAACCGCAGCTCTGCGTGGAGGACGATGACATGGGCGACCTGGTGGATGGCTGGAGAGATCTGGCTCCACGGCTTCTCTGTCAG	3101	3101
Human	GACAGCACTTCTACCGCTCACTGCGTGGAGGACGATGACATGGGCGACCTGGTGGATGGCTGGAGAGATCTGGCTCCACGGCTTCTCTGTCAG	3101	3101
E.coli	ACCCAGGGCTGGCGAGGGGTTAGGTCTACCCACGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3201	3201
Human	ACCCAGGGCTGGCGAGGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3201	3201
E.coli	AGAAGAGGGACCACGTTGCGAGCCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3301	3301
Human	AGAAGAGGGCCCCAGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3301	3301
E.coli	CTGGCAACGATGACCCGCTCCCTGCGTGGCGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3401	3401
Human	CTCCCGACAGCTGACCCGCTCCCTGCGTGGCGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3401	3401
E.coli	CTGGCAACGAGATAATGTGATCTGACCCGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3501	3501
Human	CTGGCAACGAGATAATGTGATCTGACCCGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3501	3501
E.coli	GGAGCTTCAAAAGACCTTGGAGGGGGTAAGAATGGCGTGGTCAAGACGTTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3601	3601
Human	GGAAACCCCCAAGACTCTCTCCCCAGGAAAGAATGGGGCTGTCAGGAAACGTTGGGCTGGGCTGGGCTGGGCTGG	3601	3601
E.coli	GGAGCTGGAGCTGGCTACAGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3701	3701
Human	GGAGCTGGAGCTGGCTACAGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGGGCTGG	3701	3701
E.coli	CAAGCACCTTCAGGGCAACCCACGGCAGAGAAACCTGAAATATCTGGGTTGGACGTTGCAGGCTGG	3769	3769
Human	CAAGCACCTTCAGGGCAACCCACGGCAGAGAAACCTGAAATATCTGGGTTGGACGTTGCAGGCTGG	3769	3769

DNA encoding human EGFR: comparing E.coli codon optimized sequence to human gene sequence.

E.coli	ATGAGGCCAACGGGCACGGCAGGCGCAGGATTACTGGCATTACTCGCCGCCTTCTGGCCAGCGAGCCGTGCACTGGAA	101
Human	ATCGACCTCTCGGGCACGGGCCCTGGGCTGCTGGCTGCCCTCTGGGCTCGCGAGTCGGGCTCTGGAGGAAAGAAGT	101
E.coli	CCAGCAATAAGCTGACTCAAATGGGACCTTGAGGACACTTTCTGTCCTCCACACGTTATGTCAAACACTCGAGGCTG	201
Human	CGAGTAACAAAGCTCACGCAGTGGGACTTTGAGATCATTTCTCAGCTCCAGAGGATGTCATAACTGTGAGGTGTCCT	201
E.coli	CACCTACGTTAACGTAACATCGACCTGAGCTTCCTGAAAACCATTCAGGAAGTGGCGGCTACGTTCTGATTTGTCGAA	301
Human	TACCTATGTCAGAGGAATTATGATCTTCCTTAAAGACCATCAGGAGGTGCTGGTTATGTCCTCATTCGCCCCTCA	301
E.coli	CTGGAAAAACCTGGAAATCATTCGCGGAAACATGACTATGAGAAATACCTATGGGCTGGCCGCTGCTGAGCAATTAC	401
Human	TGGAAAAACCTGGAGATCATCAGAGGAATTATGACTACGAAATTCCTATGGCTAGCAGTTATCTAACTATGAGC	401
E.coli	AGCTGCCATCGCTAACATCTGAGGGAGATTCTGCACGGTGGCTCCCTCAGCAATAACCCCTGCCCTGTCGAA	501
Human	AGCTGCCATCGAAATTTCAGGAAATCTGCATGGCCTGGCTTCAGCAACAAACCCCTGCCCTGTCGAA	501
E.coli	CGTTCTCGGACTTCTGAGCAAATGAGCACTGGACTTCAAACACCTGGGAGCTGTCAAAAGTGTGA	601
Human	AGTCAGCTGACTTCTCAGAACATGTCATGGACTTCAAACACCTGGGAGCTGTCAAAAGTGTGA	601
E.coli	GGTGGGGGGAGAGAAACTGCGAGAACATGACCATGATCTGTGACAACAGTGCAGGGCGTTGCGCGGGTTAGT	701
Human	GGTGCAGAGAGGAAACTGCGAGAACATGACCATGATCTGTGACAACAGTGCAGGGCGTTGCGCGGGTTAGC	701
E.coli	ATCAATGTCGTTGGTTCGACGGGCTCCACGTTGAGAGCGATTGTCCTGGTTAGTCCGTTAGGATAGTC	801
Human	ACCAGTGTGTCAGGCTGCAAGGGCCCCGGAGAGCAGTCCCTGGCTGCCAGAACAGTGCAGAGGACAC	801
E.coli	GATGCTGTAACACCGGACCACTATACGTTATGAGCTTATGGGAGCTTAACTATAGCTTGGGCGCTGTC	901
Human	CATGCTACAAACCCACCACTGACCATGATGTCAGGCCCCGAGGCCAAATACAGCTTGGTCCACCTGTC	901
E.coli	GTGACGGGACCATGTTAGCTGTGTCGGCGTGGGATAGCTACGAGATGGAGAAGATGGCGTTCGTTAAGT	1001
Human	GTGACAGATCACGGCTCGTGTGTCGGCGACAGCTATGAGATGGAGAAGCAGGGCTCCGCAAGTGTAAGA	1001
E.coli	GTAAGGTGTGCAACGGTATTTGGCATCGGTAATTCAAAAGACTCTCTGACCATCAATGCAACGAA	1101
Human	CAAAGTGTCTAACGGAAATAGGTATTTGGTAATTCAAAAGACTCTGACCATAAATGCTACGAA	1101
E.coli	CGATCTGCAATACCTGCGGGTGGCGTTCTGGCATTAGGGTGACTCTTCACACATACTCTCTGGATCC	1201
Human	CGATCTCCACATCTGCGGGTGGCATTAGGGTGACTCTTCACACATACTCTCTGGATCCACAGGAAC	1201
E.coli	ATCACCGGCTTCTGCTGATTCAGGCTGCGGAGGAAACCCGACCGATCTCCACGGGATTTGA	1301
Human	ATCACAGCTTCTGCTGATTCAGGCTGCGGAGGAAACCCGACCGGACCTCCATGCGCTTGTGAA	1301
E.coli	GTCAGTTTGCCTGGCGTCGCTGACCTGAAACATACCCAGCTGGGTTCCTGGCTTCTGAGCTAC	1401
Human	GTCAGTTTGCCTGGCGTCGCTGACCTGAAACATACCCAGCTGGGTTCCTGGCTTCTGAGCTAC	1401
E.coli	AAATCTGTGCTAGGCCAACACCACTAACCTGGAAAGAGCTGGCACGGGTCTGAGAAACAGCTG	1501
Human	AAATTGTGCTAGCAAAATACAAATACCTGGAAAGAACCTGGCACGGGTCTGAGAAACAGCTG	1501
E.coli	GCGACCGCTCAGGTTGTCATGCTTGTGCTAGCGGGGAGGGTTGCTGGGCTCCGGACCGGCTG	1601
Human	GCGCACAGGCCAGGTTGTCATGCTTGTGCTCCGGCGGGGAGGGCTGCTGGGCTCCGGACCG	1601
E.coli	ATGCGTTGATAAGTGTAACTGCTGGAGGGCGAACCTCGTGAATTCTGAGAGATAGCGAAC	1701
Human	ATGCGTGGAGAAGTCAACCTTCTGGAGGGTGGCCAGGGAGTTGTCAGGACTCTGAGTCAT	1701
E.coli	GAACATTAACGTTACCGGTCGGTGGAAATACCGGAGACGGGACCAACGAACTGTATCC	1801
Human	GAACATCACCTCACAGGACGGGGACCAACGAACTGTATCCAGTGTGCCCCACTGCGCTAC	1801
E.coli	GGTGGAAACAAATACTCTGGTGTTGGAAATACCGGAGACGGGGGTACCGTCTGCCACCTGTG	1901
Human	GGAGGGAAACAAACACCCCTGGTCGGAGATACCGGAGACGGGGGTACCGTCTGCCACCTAC	1901
E.coli	AGGGCTGTCCGACCAACGGCCCGAACATCCCGAGCATCGCTACCGGCATGGTCTGGGCT	2001
Human	AAGGCTGTCCGACGAAATGGGCTTAAGATCCCGTCATCGCCACTTGGCATGGTCTGGGCT	2001
E.coli	TATGGCTCTGGTCACATGTGTAACGAAACGTAAGTTGGCCCTGGGCTTGTGCAAGGAGCT	2101
Human	CATGGCAACGGGACACATCGTCTGGAGAGCCACGCGTGGGAGGCTCTGCAAGGAGCT	2101
E.coli	CAGGGCTCTGGTATCTGGAAAGAAACGAGTTCAAGAAGATCAAGGTGCTGGGAGCCGGT	2201
Human	AGGGTGAAAGGTGAAATTCCGGTTGGCATTAAGAGAGCTGCGCGAACAGCCGAAAGGCA	2201
E.coli	AAGCGTTGATAATTCCGTCGTTGGGTATTGGTCTGACCCAGCGTCCAGCTGTCAGCTG	2401
Human	CAGCGTGGAGAACCCCCCGCTGGTGTGCGCCCTGCTGGGACATCTGCGCHACCGT	2401
E.coli	TACGTGCCGACCAATAGGAAATATTGGAGCCAGTACCTGCTGAAATTGGTGGCTTC	2501
Human	TATGTCGGGAAACAAAGAACAAATATTGGTCGGAGATCTGCTGAGATCGCAAAAGG	2501
E.coli	TCCATCGGACCTGGCTCGAACGTTGTTAAACACGGACAGCACTGGCAAGATCACAG	2601
Human	TCCACCGCGACCTGGCAGCCAGGAACGTTGTTGAAACACGGCAAGCACTGGCAAG	2601
E.coli	AGATACCAACGGGGCGTAAGGTTCGGATGGCTGGGAAAGGATCTGCTGTTAC	2701
Human	AGAATACCAATGGAGGGGCGAACAGTGGCTGGGAAATGGGATGGCTGGGAAAGG	2701
E.coli	GGCGTACCGCTCTGGGAGCTGATGACTTGGTAGCAACCGGTACGACGCTTGGGCCAG	2801
Human	GGCGTACCGCTTGGGAGTTGATGACCTTGGTAGCAACAGCACTGGGCCAGGAGA	2801
E.coli	CTCAGCCGCCATTTGACCACTTGATGTCATGTCATGTCATGGTCAAGTGTGAT	2901
Human	CTCAGCGACCGATATGTCATGTCATGTCATGGTCAAGTGTGATAGACGCGAGAT	2901
E.coli	ATTCAACAAATGGCGCTGATTCGGCAACCGTACCGGCTTGGTATCCACGCTTGG	3001
Human	ATTCTCAAAATGGCCCGAGACCCCGACCGCTACCGTCTGGTATCCACGCTTGG	3001
E.coli	CTGATGGAAGAGGATAATGACCGACGCTGCGGACGAACTTACCTGAGCTAC	3101
Human	CTGATGGAAGAGGATAATGACCGACGCTGCGGACGAACTTACCTGAGCTAC	3101
E.coli	TGTTGAGCTGCTGGTCCGCAACAGAACATAGCACTGTTGCAATGCAATTGAT	3201
Human	TCCCTGAGCTCTGAGTGCAACCAGAACAAATCACCAGTGGGTTGCAATTGAT	3201
E.coli	GGCTATAGGAGGGACCGGACGGTGCACGACGAGGATTCTATCGACGACAC	3301
Human	GGCATACAGCTCACGACCCACAGGCCTGACGAGGACAGCATACGACGACAC	3301
E.coli	CGCGCTGCTAGCGTTCAACCGGGTTACCAAACTACGACGACCTCGAAGTCCG	3401
Human	CGCGCTGCTCTGTCAGGAACTCTGTCATTCACAACTACGACGACCTCGAAGT	3401
E.coli	GCAATCGGGATATCTGAAACACCGTGCAAGGGACCTCTGCGTGAATAGCAG	3501
Human	GCAACCCCGAGTATCTCAACATCGTCAAGGGACCTCTGCGTCAACAGCAG	3501
E.coli	GGACAAACCGGAGCTATCAACAGGAGCTTTCCTGGAAAGAGGCTAAGGG	3601
Human	GGACAAACCGTGAAGTACAGCAGGAGCTCTTCCCAAGGGACCAAGGGCA	3601
E.coli	GGACCGCAACOAGCGAGTTACCGGTTCCGATCGGTTCC	3634
Human	GGCCGCAACAGAGTGAATTATGGAGGAAGA	3634

## Supplementary Tables

**Supplementary Table 1. List of genes and plasmids.**

Plasmid	Vector	Gene	Tag	Resistance
pJexpress 414- EGFR	pJexpress 414	EGFR *	None	Amp
pJexpress 414- ERBB2	pJexpress 414	ERBB2 *	None	Amp
pIVEX-Δ49A1	pIVEX2.4b	Δ49A1	6xHis	Amp

pJexpress 414 is from DNA2.0; pIVEX2.4b is from Roche.

- Genes are codon optimized for *E.coli* expression. For sequence details see Supplementary Figure 7.

**Supplementary Table 2. List of antibodies.**

Antibody name	Target	Host	Comp.	Clone	Dilution
Anti-c-ErbB2/c-Neu (Ab-3)	hERBB2, C terminal	Mouse	EMD	mAb	1:1000
p-Tyr Antibody (4G10)	phosphotyrosine	Mouse	EMD	mAb	1:1000
Phospho-ErbB2 (Tyr1248) Antibody	hERBB2, phosphor-tyrosine at 1248	Rabbit	Assay Biotech	pAb	1:1000
Trastuzumab	hERBB2, juxtamembrane	Humanized	Genentech	mAb	N/A
EGFR Antibody (1005)	hEGFR, C terminal	Rabbit	Santa Cruz	pAb	1:1000
Phospho-EGF Receptor (Tyr1110) Antibody	hEGFR, phosphor-tyrosine at 1110	Rabbit	Assay Biotech	pAb	1:1000