Supplementary Information

Techno-economic assessment of cell-free synthesis of monoclonal antibodies using CHO cell extracts

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Table S1: CFPS medium components

Component	**Concentration in mixture**	***Bulk price (US $/kg)**	**Source**
HEPES KOH, pH 7.6	30 mM	113.60	Sigma-Aldrich[1]
Potassium acetate	150 mM	11.50	Sigma-Aldrich[2]
Magnesium acetate	25.9 mM	16.68	Sigma-Aldrich[3]
ATP	1.75 mM	200.00	Carbosynth[4]
CTP	0.3 mM	2900.00	Carbosynth[5]
GTP	0.3 mM	59393.72	Sigma-Aldrich[6]
UTP	0.3 mM	778.00	Carbosynth[7]
Amino acids	100 μM	34.29	Sigma-Aldrich[8]
^{14}C Leucine	9.9 dpm/pmol	68.90	Sigma-Aldrich[9]
Sodium azide	0.02%	18.36	Sigma-Aldrich[10]
Caspase inhibitor Ac-DEVD-CMK	30 μM	7100.00	Sigma-Aldrich[11]
DTT	2.5 mM	1588.00	Sigma-Aldrich[12]

*All raw material price was considered as 10% of the market selling price

S2. Analysis of monoclonal antibody demand[13]

The monoclonal antibody for lung cancer patient in the United Kingdom

- In 2011, there were 141,000 patients with lung cancer in the United Kingdom[14]
- Only 80–85% of lung cancer patients have non-small cell lung cancer[15]
- Approximately 75% of patients of non-small cell lung cancer have non-squamous non-small cell lung cancer.
- Only 40 % of non-squamous non-small cell lung cancer patients are in the stage that can be treated by Avastin.
- Five percent market penetration capacity of Avastin for treating lung cancer patients in the United Kingdom.
- Assume a dose size of 15.5 g per person per year.

$$Amount\ of\ mAb\ required\ = 141000 \times 0.85 \times 0.75 \times 0.40 \times 0.05 \times 15.5$$
$$= 27865\ g\ per\ year\ \cong 28\ kg/year$$

Reference:

Web-source for raw material prices

1. HEPES KOH: https://www.sigmaaldrich.com/catalog/product/sigma/h0527?lang=en&region=US, accessed in June 2019
2. Potassium acetate: https://www.sigmaaldrich.com/catalog/product/sigma/p1190?lang=en&region=US, accessed in June 2019
3. Magnesium acetate: https://www.sigmaaldrich.com/catalog/product/sigma/p1190?lang=en&region=US, accessed in June 2019
4. ATP, https://www.carbosynth.com/Carbosynth/Catalogs.nsf/0/631BEFB11CAE17B380

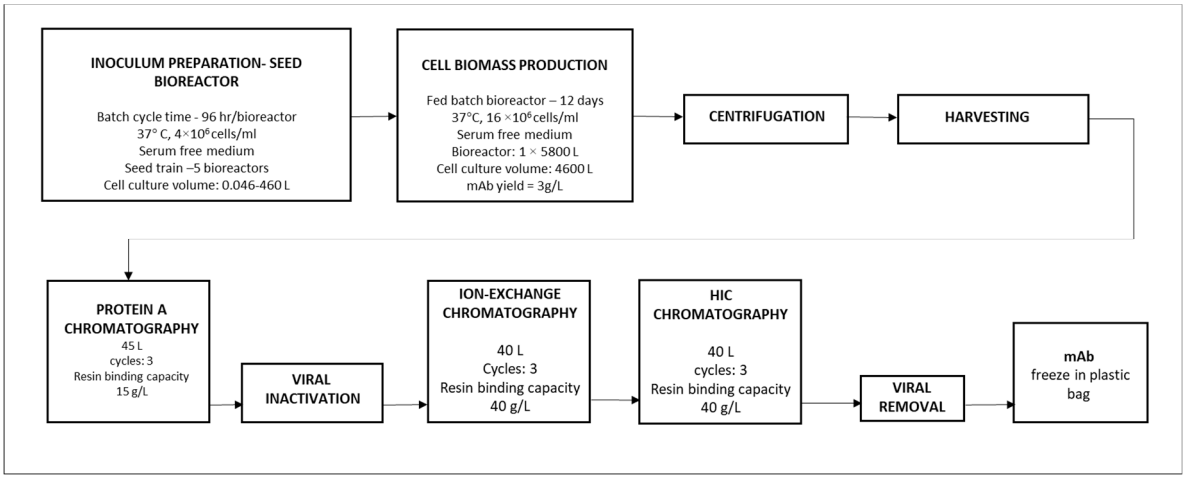
257E29004F2B8E/$FILE/Carbosynth_Nucleoside_and_Phosphoramidite_Catalogue.pdf, accessed in June 2019

5. CTP: https://www.carbosynth.com/Carbosynth/Catalogs.nsf/0/631BEFB11CAE17B380257E29004F2B8E/$FILE/Carbosynth_Nucleoside_and_Phosphoramidite_Catalogue.pdf, accessed in June 2019
6. GTP: https://www.sigmaaldrich.com/catalog/product/sigma/g8877?lang=en&region=US, accessed in June 2019
7. UTP: https://www.carbosynth.com/Carbosynth/Catalogs.nsf/0/631BEFB11CAE17B380257E29004F2B8E/$FILE/Carbosynth_Nucleoside_and_Phosphoramidite_Catalogue.pdf, accessed in June 2019
8. Amino acids: Mixture of all 20 amino acid https://www.sigmaaldrich.com/catalog/search?term=alanine&interface=All&N=0&mode=match%20partialmax&lang=en&region=US&focus=product, accessed in June 2019
9. ^{14C}Leucine, https://www.sigmaaldrich.com/catalog/product/sigma/l8912?lang=en&region=US, accessed in June 2019
10. Sodium azide,https://www.sigmaaldrich.com/catalog/product/sial/s2002?lang=en&region=US, accessed in June 2019
11. Caspase inhibitor Ac-DEVD-CMK, https://www.sigmaaldrich.com/catalog/product/mm/218750?lang=en&region=US, accessed in June 2019
12. DTT, https://www.sigmaaldrich.com/catalog/product/sial/s2002?lang=en&region=US, accessed in June 2019

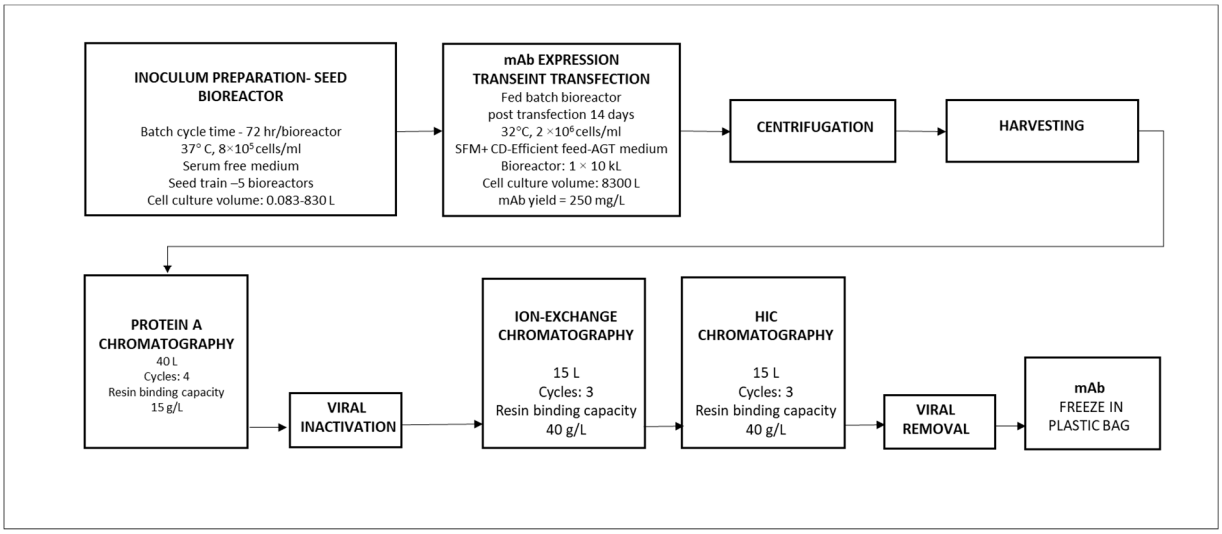
13. P. Bunnak, R. Allmendinger, S. V Ramasamy, P. Lettieri, N.J. Titchener-Hooker, Life-cycle and cost of goods assessment of fed-batch and perfusion-based manufacturing processes for mAbs, Biotechnol. Prog. 32 (2016) 1324–1335. doi:10.1002/btpr.2323.
14. British Lung Foundation. Lung Cancer Statistics. Retrieved July 18, 2016. https://statistics.blf.org.uk/lung-cancer
15. American Cancer Society. What is non-small cell lung cancer? Retrieved July 18, 2016. http://www.cancer.org/cancer/lungcancer-non-smallcell/detailedguide/non-small-cell-lung-cancerwhat-is-non-small-cell-lung-cancer.

Table S.2: Process Economic data for large-scale manufacturing via SGE and CFPS (200 kg mAb/year)

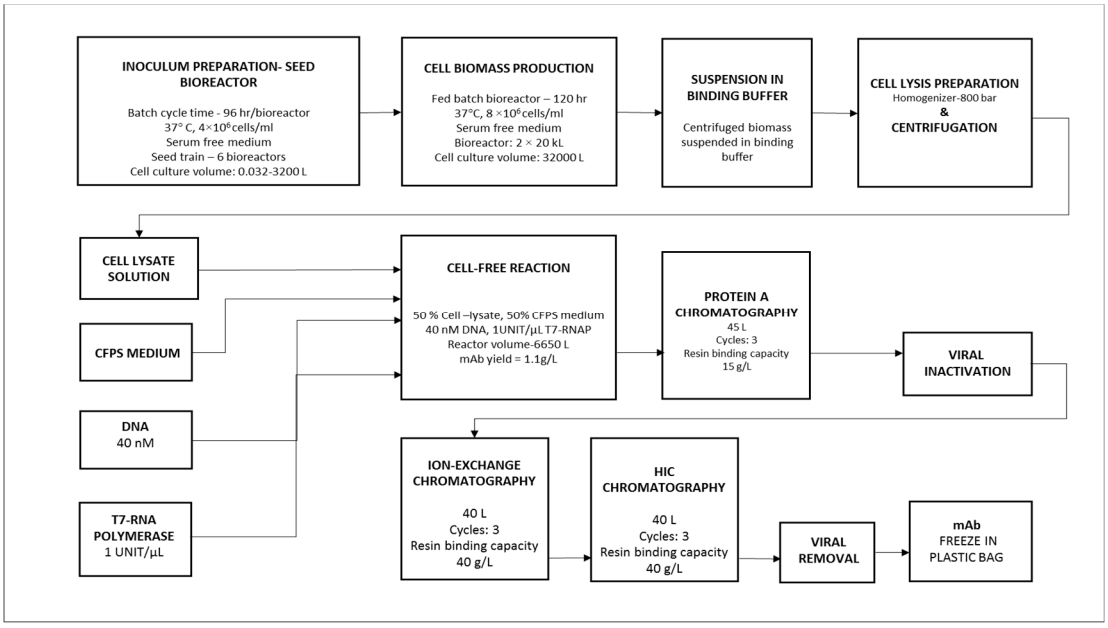
Values			Cost US $ (2018)	
Total Plant Direct Cost (DC)			**SGE**	**CFPS**
Equipment Purchase Cost	PC		7.167	10.472
Installation	IC		3.793	4.009
Process Piping	0.35 × PC		2.508	3.665
Instrumentation	0.40 × PC		2.867	4.189
Insulation	0.03 × PC		0.215	0.314
Electrical	0.10 × PC		0.717	1.050
Buildings	0.45 × PC		3.225	4.710
Yard Improvement	0.15 × PC		1.075	1.570
Auxiliary Facilities	0.40 × PC		2.867	4.190
DC			24.433	34.20
Engineering	0.25 × TPDC		6.108	8.552
Construction	0.35 × TPDC		8.551	11.959
Total Plant Indirect Cost (IC)			14.660	20.501
Total Plant Cost (TPC = TPDC+TPIC)			39.093	54.67
Contractor's Fee & Contingency (CFC)				
Contractor's Fee	0.05*TPC		1.955	2.773
Contingency	0.1*TPC		3.909	5.467
CFC			5.864	8.200
Direct Fixed Capital Cost (DFC = TPC+CFC)			**44.56**	**62.87**
Working Capital				
30 days of labour			0.36	0.56
30 days of Raw Material			0.09	47.26
30 days of Utilities			0.0013	0.01
Working Capital			0.46	47.83
Start-up	0.05*DFC		**2.25**	**3.14**
TC =DFC + Working Capital + Start-up			**47.67**	**113.85**



(a)



(b)



(c)

Figure S1: Schematic process block diagrams for mAb production by: (a) SGE (b) TGE and (c) CFPS.

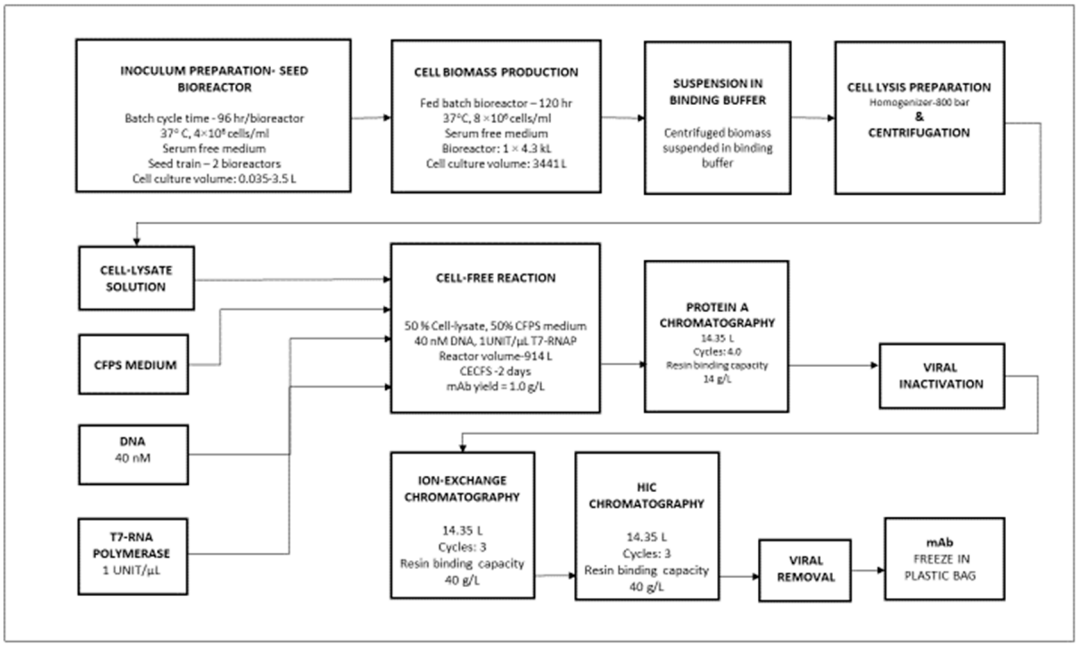


Figure S2: Schematic process block diagrams for mAb production 25kg/year by: (a) CFPS.

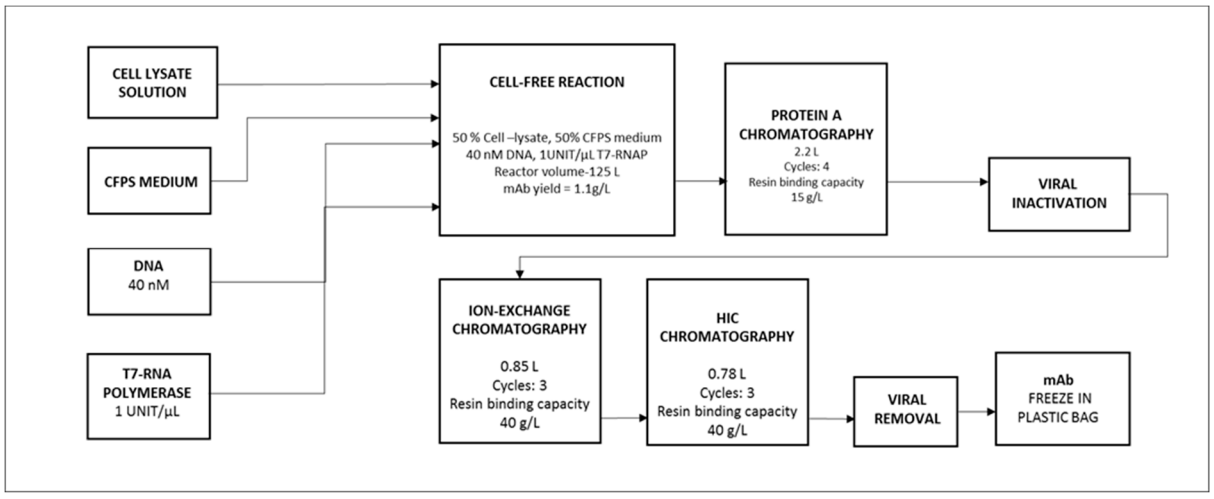


Figure S3: Process flow for personalised medicine manufacturing process

Table S3: The operating cost per batch of 1L CFPS reaction volume

Operating cost/batch	**Cost, $** (without DNA recycle, T7-RNAP, 6.9 $/5000 UNITs)	**Cost, $** (with DNA Recycle, T7-RNAP, 6.9 $/5000 UNITs)	**Cost, $** (with 80% DNA recovered T7-RNAP, 6.9 $/5000 UNITs)	**Cost, $** (with DNA recycle, T7-RNAP, 2.0 $/5000 UNITs)	**Cost, $** (with 80% DNA recovered T7-RNAP, 2.0 $/5000 UNITs)
Raw Materials	2445	1845	1965	874	994
Facility Dependent	26248	26248	26248	26248	26248
Labour-Dependent	23318	23318	23318	23318	23318
Consumables	7012	7012	7012	7012	7012
Utilities	7	7	7	7	7
Waste Treatment/Disposal	333	333	333	333	333
Total operating cost/batch	59363	58763	58883	57792	57912
Redefined operating cost (without Facility dependent cost), $/batch	33115	32515	32635	31544	31664
The mAb production cost, $/mg	43	42.27	42.38	40.96	41.12

For the assumed mAb demand of 20 g/person/year, mAb COG was estimated as below.

- Total 26 number of batches are required to produce 20 g/year
- For 1 L CFPS reaction volume, mAb produced per batch = 770 mg
- Here mAb COG is defined as Raw material cost per batch

Therefore, from TableS3,

mAb COG per batch = $2445, producing 770 mg mAb/batch

Thus, mAb COG was estimated for the assumed mAb demand for one person over year (mAb demand = 20 g/person/year) = $63,506/person/year. Figure S4 shows the mAb COG with recycling DNA and with considering 80 to 60% DNA recovery.

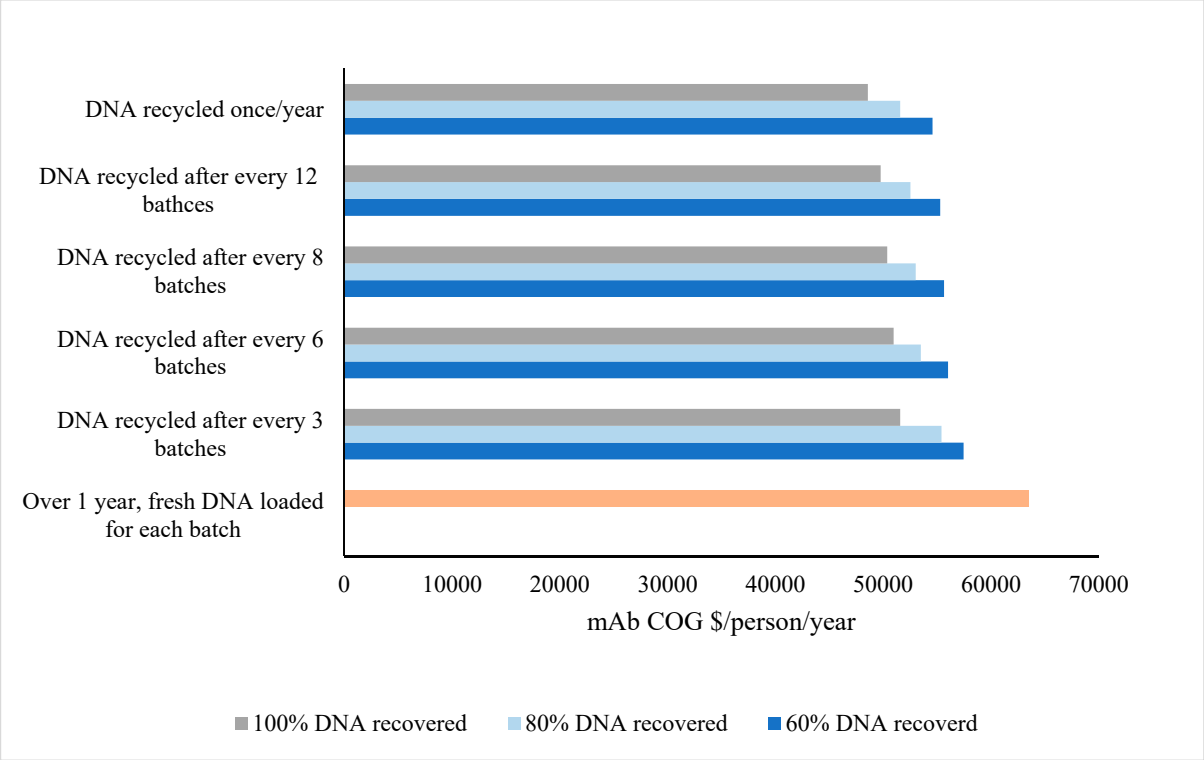


Figure S4: The effect of DNA recovery on mAb COG, $/person/year for the assumed mAb demand as 20 g mAb/person/year. (mAb COG =Raw material cost)