

Available CFTR orthologs

An emerging strategy to obtain structural information for a protein is to study orthologs, sequence homologs from different species (Lewis, Buchanan et al. 2004). This multi-species approach is based on the observation that changes in amino acid sequence, while conserving biological function, can affect protein expression and physical properties. The CFTR 3D consortium selected twelve CFTR orthologs (Table 1), plus human CFTR. Pig, ferret and mouse CFTR were included because animal models of cystic fibrosis are available for these species (Zeiber, Eichwald et al. 1995, Sun, Yan et al. 2008, Welsh, Rogers et al. 2009). For expression across yeast and mammalian platforms, genes were optimized by modifying a strategy we demonstrated to improve yield and quality of P-glycoprotein expressed in *Pichia pastoris* (Bai, Swartz et al. 2011). We compiled a hybrid codon usage table taking into account primarily human codon usage (<http://www.kazusa.or.jp/codon/>) and secondarily *Saccharomyces cerevisiae*, *P. pastoris* (Bai, Swartz et al. 2011) usage while avoiding any codons of rare prevalence in yeast. Genes were synthesized *de novo* (GeneArt, Regensburg), assembled into expression plasmids and disseminated to consortium labs for parallel expression analysis in human HEK293, BHK and CHO cells, and the yeasts *P. pastoris* and *S. cerevisiae*, and bacteria *E. coli* (subdomains). An N-terminally RGSHis₁₀-tagged Smt3 domain, shown to enhance folding and solubility of isolated CFTR NBDs (Atwell, Brouillette et al. 2010), was introduced into the first synthetic CFTR gene, killifish CFTR. For all subsequent orthologs, Smt3 was replaced by SumoStar (two amino acid substitutions, LifeSensors Inc.) to prevent cleavage by intrinsic eukaryotic Sumo proteases. SumoStar addition to gene-optimized human CFTR greatly enhanced CFTR expression compared to either wild-type or codon-optimized CFTR in *P. pastoris* and in *S. cerevisiae*, as well as in HEK cells (Hildebrandt, Zhang et al. 2014, Hildebrandt, Ding et al. 2015). The available data suggest that codon optimization and tag placement were critical to improving production of human CFTR, and likely of other orthologs as well. SumoStar addition improved both expression level and surface targeting in HEK cells. The gene-optimized, tagged CFTR in HEK membrane preparations displayed channel characteristics very similar to native CFTR, validating the approach.

Table 1: CFTR orthologs

Groups	Species	Protein identity
Placental mammalian	Human	100
	Pig	92
	Ferret	92
	Rabbit	92
	Sheep	91
	dog	90
Marsupial mammalian	Brush-tailed possum	86
Prototherian mammalian	Duckbill platypus	84
Bird	chicken	80
Placental mammalian (short life cycle)	Mouse	79
	Rat	78
Bony fish	Atlantic salmon	61
Cartilaginous fish	killifish	59

Please request DNA samples from ina.urbatsch@ttuhsc.edu
Or robert.ford@manchester.ac.uk

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