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Comparisons of Five DNA Repair Pathways Between Elasmobranch Fishes and Humans

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

Comparisons of five DNA repair pathways between two elasmobranch fishes and humans

By

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Abstract

Although DNA repair capacity has been correlated with lifespan in terrestrial vertebrate species, it remains unknown how evolutionarily conserved the process is across all vertebrate taxa. In particular, chondrichthyan fishes have lifespans that range from 3-350 years and they are evolutionarily separated from modern humans *Homo sapiens* by approximately 400 million years. We hypothesized that chondrichthyan fishes would show significant homology in nuclear excision repair (NER) genes with humans, and that the expression of NER genes will correlate with the lifespan of the respective assessed species. For this study, DNA repair gene homology and expression was performed on the nurse shark *Ginglymostoma cirratum* (n=3) and yellow stingray *Urobatis jamaicensis* (n=3). The five main NER pathways were analyzed and compared to see the differences in both elasmobranch species, then compared with human foreskin fibroblast samples (n=3). RNA sequencing was used to determine the extent of gene expression in each species, comparing the read counts in each gene and comparing between the two species. The elephant shark *Callorhinchus milii* reference genome was used to align the nurse shark and yellow stingray samples. Homology of each gene of the NER pathways was assessed by the NCBI BLAST software. Results show that the MMR pathway has all the significant genes in higher frequencies in the nurse shark than in human. Within elasmobranchs in the five DNA repair pathways, the longer-lived species (nurse shark) has a significant higher gene expression than shorter-lived species (yellow stingray). Genes involved in the NER and BER pathways showed significantly lower expression in elasmobranch than in humans. However, there were significantly higher expression of more genes for the HR and MMR pathways in elasmobranchs than in humans.

Keywords: Elasmobranch, DNA repair, Nurse shark, Yellow stingray, Longevity, , RNA sequencing.

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Introduction

Cancer is one of the top research topics all over the world because the disease affects many people. This disease is very complex and affects people in different ways. Not all animal species seem to experience cancer as humans do, especially aquatic vertebrates. Sharks and other elasmobranchs are relatively long-lived fishes, but there is only a minimal evidence reported of cancers on these species.

DNA repair mechanisms are known to be correlated with the longevity of the individual at the species level. It is well described in other species like mice, humans, naked mole rat, bats and bowhead whale. Therefore, we decided to examine the DNA repair mechanisms at the genetic level for whether elasmobranchs shared the same or similar mechanisms using two species that represent different lifespans in elasmobranch longevity.

Chondrichthyes

The Class Chondrichthyes is composed of all the cartilaginous fishes, including skates, sharks, chimeras, and rays. Chondrichthyan fishes are divided into two subclasses: the Elasmobranchii, which include the sharks, rays, and skates, and the Holocephali, the chimeras. It is believed that there are 1207 species of chondrichthyans and almost half of these are found in deep waters (below 200 m) (Cotton & Grubbs, 2015). Chondrichthyan fishes have existed for at least 485 million years, and the the elasmobranch fishes in particular are separated from humans by 400 million years of evolution (Inoue et al. 2010). The Elasmobranchii are particularly vulnerable to over-exploitation because these species tend to grow slowly, reach sexual maturity at a late age, have low fecundity, and exhibit relatively long life-spans (Stevens et al., 2000).

Longevity in elasmobranchs

Sharks and rays show a wide variety of longevity among species, with lifespans ranging from 3 to 500 years (Table 1). It is a challenge to monitor the longevity of sharks in natural oceanic habitats because of the feeding and often-complex migration patterns. One method of estimating longevity is to monitor captive-born animals, but many elasmobranchs unfortunately do not survive in captivity (Mohan et al., 2004). There are other methods to validate the age of elasmobranch, such as tag-recapture and radiocarbon

isotope dating. This last method was used in the discovery of the extreme longevity of the Greenland shark (Nielsen et al., 2016).

Table 1. Selected representative elasmobranch species with their average lifespan.

Species	Average Lifespan (years)	Reference
Yellow stingray *Urobatis jamaicensis*	8	Sulikowski (1996)
Nurse shark *Ginglymostoma cirratum*	25	Clark (1963)
Great white shark *Carcharodon carcharias*	50	Hamady et al. (2014)
Greenland shark *Somniosus microcephalus*	500	Nielsen et al. (2016)
Bull shark *Carcharhinus leucas*	35	Wintner et al. (2002)
Tiger Shark *Galeocerdo cuvier*	50	Branstetter et al. (1987)
Whale shark *Rhincodon typus*	80	Hsu et al. (2014)
Spiny digfish *Squalus acanthias*	75	Cailliet et al. (2001)
Lemon shark *Negaprion brevirostris*	25	Smith et al. (1998)
Bonnethead shark *Sphyrna tiburo*	124	Carlson & Parson (1997)
Sandbar shark *Carcharhinus plumbeus*	34	Andrews et al. (2011)
Blacktip shark *Carcharhinus limbatus*	124	Compagno (1984)

Sharks and Cancer

Sharks have long been harvested in part for the production of cartilage extracts, which are believed to be able to cure or prevent cancer. This belief has both a serious impact on shark populations and resulted in the delay of effective treatments for some cancer patients (Ostrander, 2004). The notion that sharks do not get cancer was first discussed by Lane in 1992 in a book titled "Sharks Don't Get Cancer," followed by another book four years later, titled "Sharks Still Don't Get Cancer." The premises of these books have been found to be false. Marine biologists who study elasmobranchs have discovered that sharks do indeed get cancer (Ostrander, 2004; Finkelstein, 2005). For example, Robbins et al. (2014) reported proliferative lesions in the white *Carcharodon carcharias* and bronze whaler *Carcharhinus brachyurus* sharks, including the possibility of tumors on both of the animals (Figure 1).

A more recent paper by Marra et al. (2017) provides a second perspective of protection against cancer in sharks by immune surveillance and subsequent destruction of cancerous cells in the body, which could be complementing known mechanisms of deoxyribonucleic acid (DNA) repair. This paper compared the heart tissue of seven species (four elasmobranch and three teleost) using RNA sequence analysis, trying to identify genetic similarities. The comparisons were made by clustering the gene expression. The results provided the first multi-taxa, transcriptomic-based between teleost and elasmobranch.

Many of the tumors in sharks appear to be malignant, but also seem to behave less aggressively and do not metastasize as often as in mammalian species (Martineau & Ferguson, 2006). A case of sarcoma in sharks was recently discovered, in which an Arabian carpet shark *Chiliscyllium arabicum* was caught with a superficial ulcerated mass on the left lateral trunk at the level of the second dorsal fin. There was no evidence of metastasis of the tumor and an unusually dark color of the liver is believed to be consistent with hepatocellular atrophy (Camus et al., 2017). Brunnschweiler et al. (2017) documented the growth progression of a proliferation through a 7-year period (2010-2017) of a bull shark *Carcharhinus leucas* (Figure 3). The lesions on this shark were due to injuries obtained from prior interactions with fishing gear, and they appear to be showing proliferative gingivitis and cellulitis with necrosis, resulting in the deformation of the lower jaw cartilage.

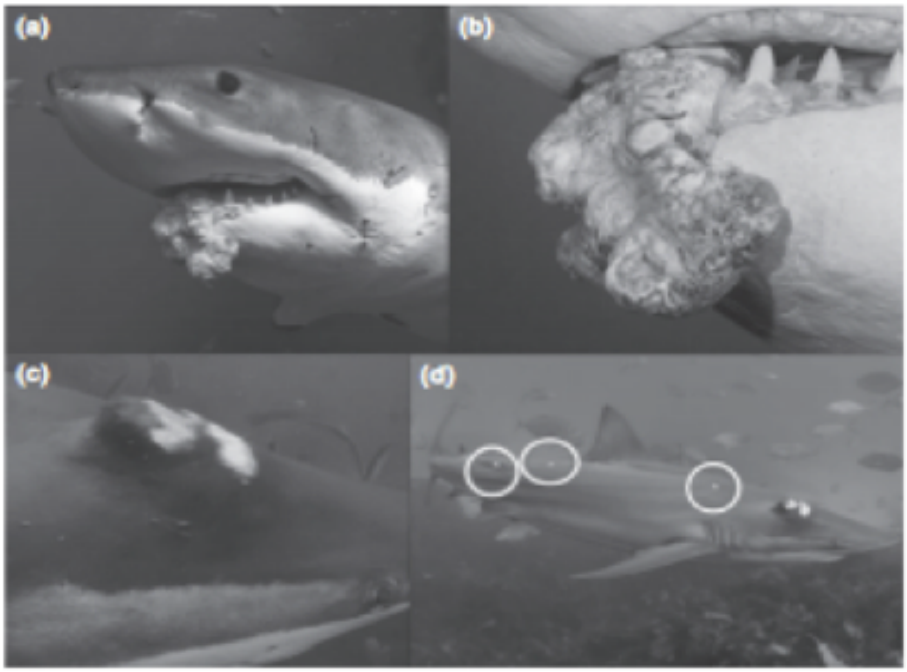

Figure 1. First Neoplastic Lesions Reported in Wild Sharks. A) and B) is a white shark *Carcharadon carcharias* and C) and D) is a bronze whaler shark *Carcharhinus brachyurus*. These are the first neoplastic lesions formally reported in the scientific literature for wild sharks. The white shark has a neoplasm on the lower jaw, which was either missing teeth or the teeth were overgrown by the mass. The bronze whaler shark has neoplasm lesions on the top of the head and along the dorsal surface of the body (white circles). Source: Robbins et al. (2014).

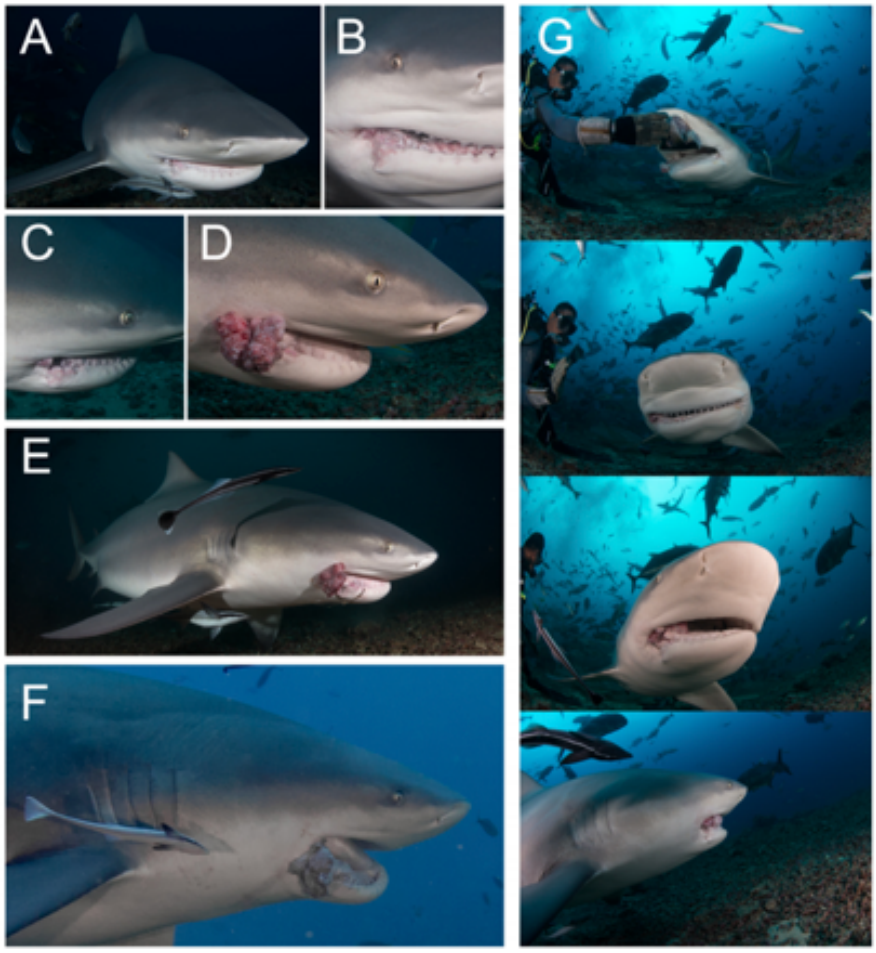

Figure 2. Growth Progression of a Proliferation. Growth progression of a proliferation through a 7-year period. A,B *Carcharhinus leucas* photographed on January 10, 2010, C June 7, 2011, D March 24, 2013, E April 26, 2014, F June 3, 2016. The healed injury (broken jaw) is visible in A–F. G. Sequence showing the proliferation dangling inside the mouth when the shark takes a fish head from the feeder. Photographs taken on March 24, 2013. Source: Brunnschweiler et al. (2017).

DNA Repair in Mammals

A positive correlation between DNA repair gene expression and aging has been previously described for mammals by Kraemer et al. (1994). DNA repair genes suppress cancer by maintaining the integrity of the DNA code. Other studies have correlated the higher incidence of observed cancer to a reduced expression of various DNA repair genes (e.g., Garfinkel & Bailis, 2002; Broustas & Lieberman, 2014). The DNA repair comparison of extreme lifespan in mammals was described by MacRae et al. (2015) using RNA-sequencing. The comparison is between human *Homo sapiens* (maximum lifespan: 120 years), naked mole rats *Heterocephalus glaber* (30 years), and mice *Mus musculus* (3 years). The results show that the longer-lived human and naked mole rats have genomes with a higher expression of DNA repair genes. MacRae et al. (2015) therefore concluded that DNA repair is a system that is closely associated with lifespan longevity. Because elasmobranch fishes have extremely varied lifespans, the taxa provides additional opportunities to assess correlations between gene repair expression and longevity across phylogenetic divisions.

DNA Repair Mechanisms

DNA is the hereditary material in almost all organisms. The loss of DNA repair in mammals is caused by increased genomic instability, in which replication errors result in additional copies of some genes. This instability can be the results of either endogenous or exogenous exposure, which can cause DNA damage. Ultimately, the cell becomes malignant when many mutations occur and accumulate in the genetic code, including transient changes (including genomic imbalances) in the DNA that act like mutations. When the growth of cells is not controlled, a tumor occurs. There are five known pathways of DNA repair, which will each be addressed in turn: nucleotide excision repair, base excision repair, mismatch repair, homologous recombination, and non-homologous end joining (Altieri et al., 2008).

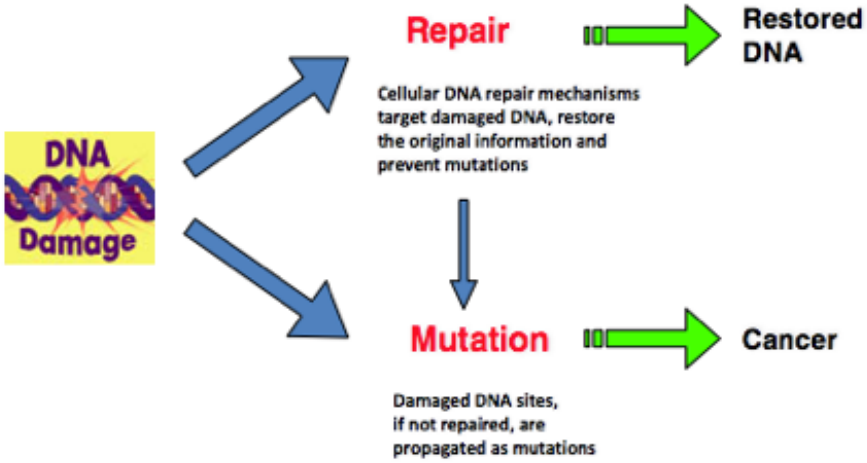

Figure 3. General DNA damage pathways. When the DNA is damaged, the body has an efficient but complex mechanism to repair this damage, called DNA repair mechanisms. If this mechanism fails, it will lead to damage carried forward to subsequent generations of cells, the final result of which might manifest as cancer due to the accumulation of these mutations. Image source: Homood As Sobei, 2017.

Homologous recombination

Homologous recombination (HR) is one of the main major pathways that applies with double-stranded DNA breaks and interstrand crosslinks caused by ionizing radiation. A total of 31 genes are known to be necessary for the repair of the damaged lesions through HR (Krejci et al., 2012). However, HR is a very efficient pathway at repairing double-strand breaks and is considered an error-free mechanism (Figure 4, Table 2). The HR pathway uses sequence homology in the undamaged sister chromatid as a guide to replace the sequences surrounding the breakpoint. The HR process is initiated is by removing a section on the 5' end of the breakpoint and generating a 3' end single strand that overhangs. This overhang looks for sequence homology on the sister chromatid. When the sequence is located, the single-stand overhand invades the sister chromatid and forms a DNA heteroduplex, called the D-loop. The 3' end overhang is used in the 3' end overhang to extend both stands. At the end, the D-loop is taken apart and the newly synthesized ends are brought together and religated. The original DNA sequence is there by restored back to double helical structure (Jasin & Rothstein, 2013).

Table 2. Homologous Recombination Genes and Function. Gene names in red font indicate human *Homo sapiens* genes not found in the elephant shark *Callorhinchus milii* genome.

GENE	FUNCTION	HOMOLOGY (%)	REFERENCES	GENE	FUNCTION	HOMOLOGY (%)	REFERENCES
ATM	Serine- protein kinase ATM Serine/threonine protein kinase which activates checkpoint signaling upon double strand breaks (DSBs), apoptosis and genotoxic stresses such as ionizing ultraviolet A light (UVA), thereby acting as a DNA damage sensor	76	(Zhang et al., 2004)	**RAD51**	Double-stranded DNA breaks arising during DNA replication or induced by DNA-damaging agents	82	(Masson et al., 2001)
BLM	Bloom syndrome protein ATP-dependent DNA helicase that unwinds single- and double-stranded DNA in a 3'-5' direction	74	(Langland et al., 2002)	**RAD51AP1**	Rad51-associated protein 1 Cooperates with PALB2 in promoting of D-loop formation by RAD51	81	(Kovalenko et al., 1997)
BRCA1	Breast cancer type 1	86	(Lorick et al., 1999)	**RAD51B**	DNA repair protein RAD51 homolog 2	71	(Masson et al., 2001)

	susceptibility protein E3 ubiquitin-protein ligase that specifically mediates the formation of 'Lys-6'-linked polyubiquitin chains and plays a central role in DNA repair by facilitating cellular responses to DNA damage				Double-stranded DNA breaks arising during DNA replication or induced by DNA-damaging agents		
BRCA2	Breast cancer type 2 susceptibility protein Involved in double-strand break repair. Binds RAD51 and potentiates recombinational DNA repair by promoting assembly of RAD51 onto single-stranded DNA (ssDNA).	79	(Hussain et al., 2004)	**RAD51C**	DNA repair protein RAD51 homolog 3 Double-stranded DNA breaks arising during DNA replication or induced by DNA-damaging agents.	76	(Sage et al., 2004)
DMC1	Meiotic recombination protein DMC1/LIM15 homolog	79	(Kinebuchi et al., 2004)	**RAD51D**	DNA repair protein RAD51 homolog 4 double-stranded DNA breaks arising	73	(Masson et al., 2001)

	Meiotic recombination, specifically in homologous strand assimilation				during DNA replication or induced by DNA-damaging agents		
EME1	Crossover junction endonuclease EME1 Interacts with MUS81 to form a DNA structure-specific endonuclease with substrate preference for branched DNA structures with a 5'-end at the branch	72	(Oegruenc & Sancar., 2013)	**RAD52**	DNA repair protein RAD52 homolog genetic recombination and DNA repair by promoting the annealing of complementary single-stranded DNA and by stimulation of the RAD51 recombinase	80	(Park et al., 1996)
FSBP	Fibrinogen silencer-binding protein Transcriptional repressor that down-regulates the expression of the fibrinogen gamma chain	65	(Lau et al., 2010)	**RAD54B**	DNA repair and recombination protein RAD54B Involved in DNA repair and mitotic recombination	66	(Miyagawa et al., 2002)
MRE11A (MRE11)	Double-strand break repair protein MRE11 Double-strand break (DSB) repair, DNA recombination,	70	(de Jager et al., 2001)	**RAD54L**	DNA repair and recombination protein RAD54-like Involved in DNA repair	76	(Swagemakers et al., 1998)

	maintenance of telomere integrity and meiosis				and mitotic recombination		
NBN	Nibrin cellular response to DNA damage and the maintenance of chromosome integrity.	71	(Stiff et al.,2005)	**RAP1**	Rap1 GTPase-activating protein 1 GTPase activator for the nuclear Ras-related regulatory protein RAP-1A (KREV-1), converting it to the putatively inactive GDP-bound state	75	(Jeyaraj et al., 2012)
POLD1	DNA polymerase delta subunit 1 High fidelity genome replication, including lagging strand synthesis and repair.	80	(Li et al., 2006)	**SHFM1 (SEM1)**	26S proteasome complex subunit SEM1 Maintenance of protein homeostasis by removing misfolded or damaged proteins, which could impair cellular functions, and by removing proteins whose functions are no longer required	84	(Sone et al., 2004)

POLD2	DNA polymerase delta subunit 2 High fidelity genome replication, including in lagging strand synthesis and repair	80	(Li et al., 2006)	**UBE2N**	Ubiquiting-conjugating enzyme E2 N Error-free DNA repair pathway and contributes to the survival of cells after DNA damage	82	(Hofmann & Pickart, 1999)
POLD3	DNA polymerase delta subunit 3 High fidelity genome replication, including in lagging strand synthesis, and repair	76	(Li et al., 2006)	**XRCC2**	DNA repair protein XRCC2 Repair chromosomal fragmentation, translocations and deletions	68	(Masson et al., 2001)
RAD50	DNA repair protein RAD50 Component of the MRN complex, which plays a central role in double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis	78	(de Jager et al., 2001)	**XRCC3**	DNA repair protein XRCC3 Repair chromosomal fragmentation, translocations and deletions	81	(Sage et al., 2004)
DSS1	26S proteasome complex subunit SEM1		(Zhang et al., 2013)	**RAD51L1**	DNA repair protein RAD51 homolog 2		(Masson et al., 2001)

MMS4L	Crossover junction endonuclease EME1	-	**RAD51L3**	DNA repair protein RAD51 homolog 4	(Masson et al., 2001)
POLD4	DNA polymerase delta subunit 4	(Li et al., 2006)			

Non-homologous end joining

Non-homologous end joining (NHEJ) is another mechanism that repairs a break of double-stranded DNA. The NHEJ pathway is faster than the other pathways and does not require a homologous template from the sister chromatid, as does HR. However, NHEJ is an error-prone mechanism, which does not attempt to repair the sequence around the break, but rather simply repairs the break itself. The NHEJ pathway has about 20 gene products (Figure 4, Table 3). NHEJ is initiated by recognizing the exposed end of double-stranded break and forming a ring-shaped structure that encircles the damaged area, allowing the exposed ends to be tethered to each other. The ends are then ligated by either removing or modifying a group of nucleotides, any existing gaps are filled with the new synthesized nucleotides, and the breaks are sealed (Davis & Chen, 2013; Weterings & Chen, 2008).

Table 3. Non-Homologous End Joining Genes and Function. Gene names in red font indicate human *Homo sapiens* genes not found in the elephant shark *Callorhinchus milii* genome.

GENE	FUNCTION	HOMOLOGY (%)	REFERENCES	GENE	FUNCTION	HOMOLOGY (%)	REFERENCES
SLC23A3	Solute carrier family 23 member 3 Protein coding gene	78	(Zhao et al., 2010)	**POLL**	DNA Polymerase lambda	72	(Aoufouchi et al., 2000)
XRCC6BP1 (ATP23)	Mitochondrial inner membrane protease ATP23 homolog Subunit of DNA dependent protein kinase for Double-strand break repair	75	(Zen et al., 2007)	**POLA1**	DNA polymerase alpha catalytic subunit Initiation of DNA replication	78	(Dantzer et al., 1998)
APLF	Aprataxin and PNK- like factor Nuclease involved in single-strand and double-strand DNA break repair	66	(Kanno et al., 2007)	**RAD50**	DNA repair protein RAD50 Component of the MRN complex, which plays a central role in double-strand break	78	(de Jager et al., 2001)

					(DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis		
XRCC4	X-ray repair cross-complementing protein 4 Enhances the binding of LIG4 to DNA. The LIG4-XRCC4 complex is responsible for the NHEJ ligation step	73	(Li et al., 1995)	**XRCC6**	X-ray repair cross-complementing protein 6 Single-stranded DNA-dependent ATP-dependent helicase, involved in chromosome translocation	83	(Tuteja et al.,1994)
LIG4	DNA ligase 4 A ligase that is part of the LIG4-XRCC4 complex is	72	(Grawunder et al., 1998)	**PRKDC**	DNA-dependent protein kinase catalytic subunit Serine/threonine-protein kinase that acts as a	78	(Yavuzer et al., 1998)

	responsible for the NHEJ ligation step				molecular sensor for DNA damage		
NHEJ1	Non-homologous end-joining factor 1 Double-strand break (DSB) repair and V(D)J recombination Bridges DNA to other proteins to aid in ligation	96	(Chusseval et al., 2006)	**XRCC5**	X-ray repair cross-complementing protein 5 Single-stranded DNA-dependent ATP-dependent helicase. Has a role in chromosome translocation.	71	(Tuteja et al.,1994)
DCLRE1C	Protein artemis V(D)J recombination is initiated by the lymphoid specific RAG endonuclease complex	76	(Mouhous et al., 2010)	**OAZ1**	Ornithine decarboxylase antizyme 1 ATP binding	87	(Lin et al., 2002)
MRE11A (MRE11)	Double-strand break repair protein MRE11	70	(de Jager et al., 2001)	**PHF1**	PHD finger protein 1		(Cao et al., 2008)

	Double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis				
PNKP	Bifunctional polynucleotide phosphatase/kinase	(Jilani et al., 1999)	**SETMAR**	Histone-lysine N-methyltransferase SETMAR	(Beck et al., 2008)
PRPF19	Pre-mRNA-processing factor 19	(Mahajan & Mitchell, 2003)			

Base excision repair

Base excision repair (BER) plays an important role in preventing mutations associated with 8-oxoguanine, which is a product of oxidative damage to the DNA. It only affects one DNA strand, this pathway recognizes and fixes the non-helical-distortions. If the damages are not repaired, there is an increased risk of mismatching in DNA replication, thereby causing an integration of incorrect nucleotides and also mutations (Figure 4, Table 4). This pathway has about 26 active genes. The BER process starts with the enzymatic reactions that are controlled by DNA glycosylases. These DNA glycosylases recognize and replace the damaged nucleotide, and this causes abasic sites. The abasic sites are cleaved by apurinic/apyrimidinic endonucleases, which lead to a generation of a single-strand breaks. The breaks are synthesized by either the long-patch pathway (in which 2-10 nucleotides around the damaged nucleotide are replaced) or the short-patch pathway (in which only a single damaged nucleotide is replaced) (David et al., 2007; Zharkov, 2008).

Table 4. Base Excision Repair Genes and Function. Gene names in red font indicate human *Homo sapiens* genes not found in the elephant shark *Callorhinchus milii* genome.

GENE	FUNCTION	HOMOLOGY (%)	REFERENCES	GENE	FUNCTION	HOMOLOGY (%)	REFERENCES
NEIL1	Endonuclease VIII-like DNA glycosylase 1 Recognition and removal of damaged bases Excises oxidized pyrimidines	73	(Wilson, 2017)	**UNG**	Uracil-DNA glycosylase Excises uracil residues from the DNA	75	(Wilson, 2017)
POLE2	DNA polymerase epsilon 2 DNA repair and replication	76	(Li et al., 1997)	**SMUG1**	Single-strand-selective monofunctional uracil-DNA glycosylase 1 Recognition and initiation of base excision	70	(Haushalter et al., 1999 & Wilson, 2017)
POLB	DNA polymerase beta Repair polymerase	77	(Bennett et al., 1997)	**POLE**	DNA polymerase epsilon DNA repair and replication	86	(Post et al., 2003)

POLD1	DNA polymerase delta 1 High fidelity genome replication, including lagging strand synthesis and repair.	80	(Li et al., 2006)	**LIG3**	DNA ligase 3 Correct defective DNA strand-break repair and sister chromatid exchange following treatment with ionizing radiation and alkylating agents.	74	(Lakshmipathy,& Campbell, 1999)
NEIL3	Endonuclease VIII-like DNA glycosylase 3 Recognition of lesions in ssDNA Excises oxidized purines	73	(Wilson, 2017)	**POLD3**	DNA polymerase delta 3 High fidelity genome replication, including in lagging strand synthesis, and repair	76	(Li et al., 2006)

NTHL1	Endonuclease III Bifunctional DNA N-glycosylase with associated apurinic/apyrimidinic (AP) ligase function that catalyzes the first step of BER AP lyase	72	(Aspinwall et al., 1997 & Wilson., 2017)	**MPG**	3-Methyladenine-DNAglycosylase I Hydrolysis of the deoxyribose N-glycosidic bond to excise 3-methyladenine Methylpurine DNA glycosylase	72	(Chakravarti et al.,1991 & Wilson., 2017)
TDG	Thymine DNA glycosylase Active DNA demethylation	77	(Neddermann et al., 1996)	**POLD2**	DNA polymerase delta 2 High fidelity genome replication, including in lagging strand synthesis and repair	80	(Li et al., 2006)

POLE4	DNA polymerase epsilon 4 Polymerase epsilon carries out replication and/or repair function.	78	(Li et al., 2000)	**PARP1**	Poly(ADP-ribose) polymerase 1 Catalyzing the poly(ADP-ribosyl)ation of a limited number of acceptor proteins involved in chromatin architecture and in DNA metabolism	77	(Kanno et al., 2007)
OGG1	8-OxoG-DNA DNA repair enzyme that incises DNA at 8-oxoG residues	86	(Wilson., 2017)	**POLE3**	DNA polymerase epsilon 3 High fidelity genome replication, including in lagging strand synthesis and repair	80	(Li et al., 2006)

APEX	AP endonuclease	(Wilson, 2017)	**XRCC1**	DNA repair protein xrcc1	(Hoch et al., 2017)
FEN1	5'-flap endonuclease 1	(Wilson, 2017)	**LIG1**	DNA ligase Leucine-rich repeats and immunoglobulin-like domains protein 1	(Wilson, 2017)
MIR631	Post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs	(Horikawa et al., 2008)	**NEIL2**	Wxcises oxidized pyrimidines Endonuclease 8-like 2	(Wilson, 2017)
PCNA	involved in the control of eukaryotic DNA replication by increasing the polymerase's processability	(Burkovics et al., 2009)	**POLD4**	DNA polymerase delta subunit 4 High fidelity genome replication and repair	(Li et al., 2006)

Mismatch repair

Mismatch repair (MMR) is a post-replication single stand pathway. During DNA replication, MMR removes mis-incorporated bases that break free and DNA polymerase proofreads the strand. This pathway also corrects insertion or deletion loops that can happen during replication (Figure 4, Table 5). There are around 35 gene products that are involved in MMR. After replication, MMR proteins recognize the DNA mismatches immediately. Meanwhile the newly synthesized strand, which is the daughter strand, can still be distinguished. Mismatch repair can also excise several nucleotides around the damaged site leaving a gap. The gap is then to be filled with the newly synthesized segment by the parental strand as a template (Fukui, 2010; Li, 2008).

Table 5. DNA Mismatch Repair Genes and Function. Gene names in red font indicate human *Homo sapiens* genes not found in the elephant shark *Callorhinchus milii* genome.

GENE	FUNCTION	HOMOLOGY (%)	REFERENCES	GENE	FUNCTION	HOMOLOGY (%)	REFERENCES
ABL1	Tyrosine-protein kinase ABL1 linked to cell growth and survival such as cytoskeleton remodeling in response to extracellular stimuli, cell motility and adhesion, receptor endocytosis, autophagy, DNA damage response and apoptosis.	81	(Yuan et al., 1997)	**MUTYH**	Involved in oxidative DNA repair Adenine DNA glycosylase	77	(Ontsubo et al., 2000)
AXIN2	Axin-2 Down-regulates beta-catenin	73	(von Kries et al., 2000)	**PMS1**	PMS1 protein homolog 1	78	(Leung et al., 2000)
BLM	Bloom syndrome protein ATP-dependent DNA helicase that unwinds	74	(Langland et al., 2002)	**PMS2**	Mismatch repair endonuclease PMS2	80	(Kadyrov et al., 2006)

	single- and double-stranded DNA in a 3'-5' direction						
EXO1	Exonuclease 1 Excise mismatch-containing DNA tracts directed by strand breaks located 5’ or 3’ to mismatch	77	(Sun et al., 2002)	**POLD3**	DNA polymerase delta subunit 3 High fidelity genome replication, including in lagging strand synthesis and repair	76	(Li et al., 2006)
MBD4	Methyl-CpG-binding domain protein 4	76	(Bellacosa et al., 1999)	**PRKCZ**	Protein kinase C zeta type	85	
MLH1	DNA mismatch repair protein Mlh1	85	(Kadyrov et al., 2006)	**RCCD1**	RCC1 domain-containing protein 1 Transcriptional repression of satellite repeats	70	(Marcon et al., 2014)

MLH3	DNA mismatch repair protein Mlh3	75	(Cannavo et al., 2005)	**RECQL**	ATP-dependent DNA helicase Q1 Repair of DNA that is damaged by ultraviolet light or other mutagens	78	(Puranam & Blackshear., 1994)
MRE11	Double-strand break repair protein MRE11 Double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis	70	(de Jager et al., 2001)	**RPA1**	Replication protein A 70 kDa DNA-binding subunit	75	(Lin et al., 1997)
MSH2	DNA mismatch repair protein Msh2 Component of the post-replicative DNA MMR	73	(Blackwell et al., 1998)	**TDG**	G/T mismatch-specific thymine DNA glycosylase Active DNA demethylation	77	(Neddermann et al., 1996)

MSH3	DNA mismatch repair protein Msh3 Component of the post-replicative DNA mismatch repair system	79	(Leonard et al., 1998)	**TP73**	Tumor protein p73 apoptotic response to DNA damage	72	(Kaelin., 1999)
MSH4	DNA mismatch repair protein Msh4 Involved in meiotic recombination.	79	(Leonard et al., 1998)	**YBX1**	Nuclease-sensitive element-binding protein 1 Mediates pre-mRNA alternative splicing regulation.	83	(Chen et al., 2000)
MSH6	DNA mismatch repair protein Msh6 Component of the post-replicative DNA mismatch repair system	69	(Leonard et al., 1998)	**PMS2P4**	-		-

ANKRD17	Ankyrin repeat domain-containing protein 17 Plays a pivotal roles in cell cycle and DNA regulation	(Menning & Kufer., 2013)	**PMS2P5**	Putative postmeiotic segregation increased 2-like protein 5	-
APEX1	DNA-(apurinic or apyrimidinic site) lyase apurinic/apyrimidinic endodeoxyribonuclease 1	(Robson & Hickson., 1991)	**POLR2J2**	DNA-directed RNA polymerase II subunit RPB11-b1	-
MSH5	MutS protein homolog 5 Meiotic recombination processes	(Guo et al., 2017)	**PRKCG**	Protein kinase C gamma type	-
PMS2P1	Putative postmeiotic segregation increased 2-like protein 1	-	**TP53**	Cellular tumor antigen p53	(Lee et al., 2018)
PMS2P2	Putative postmeiotic segregation increased 2-like protein 2	-	**TREX1**	Three-prime repair exonuclease	(Mazur & Perrino., 1999)

PMS2P3	Putative postmeiotic segregation increased 2-like protein 3	-		

Nucleotide excision repair

Nucleotide excision repair (NER) is also called long-patch repair (Latimer & Kelly, 2014). The NER pathway is responsible for the correction of damage in the DNA helix, which repairs any single-stranded bulky adduct, helix-distorting lesion. Specifically, UV-induced 6-4 photoproducts and cyclobutane pyrimidine dimers are remediated by this pathway (Figure 4, 5). There are 20 canonical genes in this pathway, and mutations in NER-related genes cause a rare disease called *xeroderma pigmentosum*. People with this disease die from cancer, most often from skin cancer at a very young age due to a defect in DNA repair. However, if they live longer, they develop and often die of internal cancer (de Boer & Hoeijmakers, 2000). The loss of the NER repair mechanisms is being examined in current cancer research since its initial discovery in breast cancer. (Latimer et al., 2010)

NER Subpathways

Humans can repair with both actively transcribed and non-transcribed gene areas, but mice can only repair actively transcribed genes (Murad et al.,1995). There are two NER subpathways that humans use to deal with NER-specific DNA damages: transcriptional-coupled repair (TC-NER) and global genomic repair (GG-NER). These damages occur in the first step of the NER process, involving recognition, it is the only step that it is different between the two subpathways and the rest of the process is the same. (Figure 5) (Scharer, 2013; Spivak, 2015).

The TC- NER pathway repairs lesions located in actively transcribed genes, while the GG-NER pathway removes lesions from the rest of the genome. Transcriptional-coupled NER is known to delete damages such as cyclobutene pyrimidine dimers more efficiently and in a higher rate than global genomic NER (Bohr et al., 1985). Within the same gene, DNA lesions were performed faster in the transcribed strand than in the non-transcribed strand (Gao et al., 1994). Actively transcribed genes play a role in numerous cellular processes, might be why it is faster to remediate. The majority of the NER-specific DNA lesions are removed by GG-NER because most of the genome is non-transcribed. Global genomic NER can also repair damage that is in the actively transcribed reasoning if there is a deficit in the transcriptional-coupled NER (van Hoffen et al., 1995).

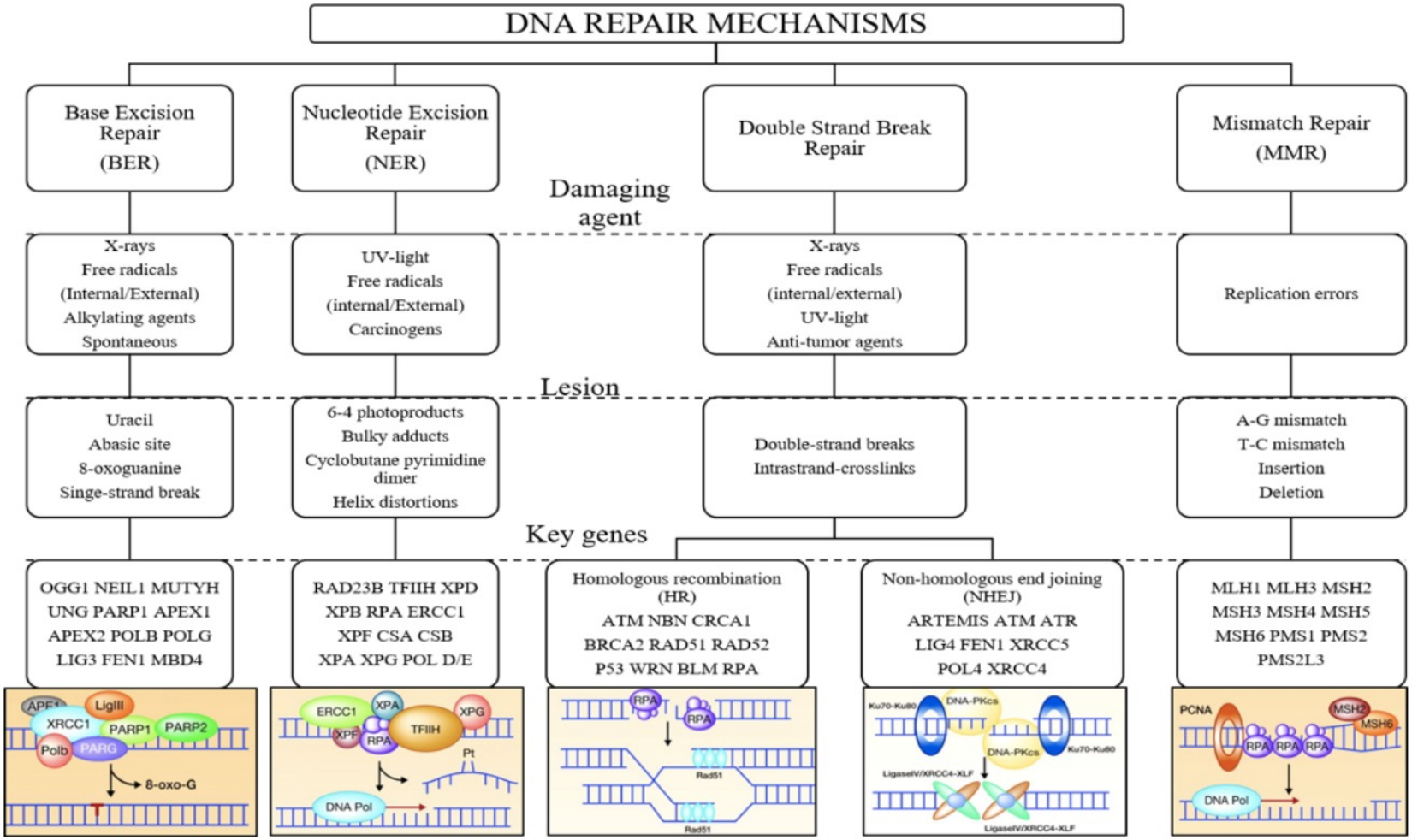

Figure 4. The 5 Major DNA Repair Pathways. Base excision repair, nucleotide excision repair, double strand break repair, and mismatch repair are the 5 major pathways. Homologous end-joining and non-homologous end joining are two different pathways that repair double strand breaks. Adapted with permission from Jalal et al. (2011).

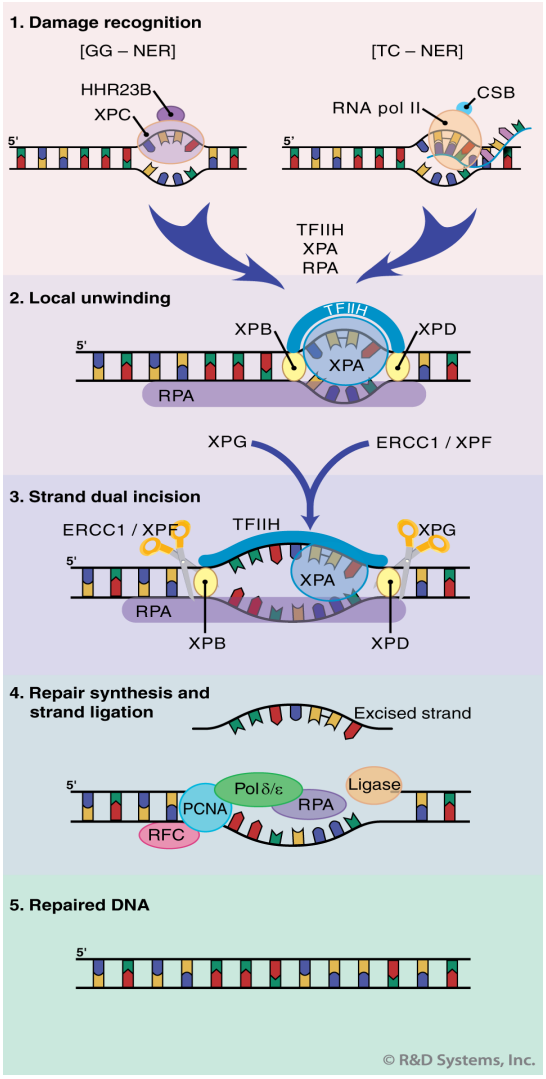

Figure 5. Mechanistic summary of nucleotide excision repair. 1. recognition of the damage through global genomic or transcription coupled damage recognition proteins; 2. unwinding the DNA around the damage to allow repair; 3. incision and excision of a 27-29-nucleotide segment around the damage; 4. resynthesis of new nucleotides to fill the gap and ligation of the nicks around the newly synthesized DNA segment; and 5. DNA damage is fully repaired. Image used with permission of Research & Development Systems Catalog.

Longevity and NER

Studies have examined the connection between longevity in mammalian species and NER, showing a correlation between the length of an animal lifespan and the efficiency of NER on the total genome. It is known that humans have a robust NER, both transcriptional-coupled and global genomic repair (Cleaver et al., 1995& MacRae et al., 2015), but few aquatic species have been evaluated with respect to DNA repair (Kienzler et al., 2013). Because elasmobranchs are relatively long-lived fishes, one hypothesis to explain their longevity could be a robust DNA repair (Kneebone, 2008; McFarlane & Beamish, 1987).

RNA Sequencing

This research was performed using a new technology called RNA sequencing, which is one of the newest next generation sequencing technology using synthesis methodology. This technology uses Sanger sequencing, a chain termination method of sequencing in combination with the restriction of the template on a glass surface or nano beads. Sanger sequencing allows multiple cycles of addition of nucleotides for detection of incorporation and the sequence of RNA (Sanger et al., 1977; Weber, 2015). Figure 6 shows the workflow of a sequencing run.

The process of the RNA samples for sequencing begins with library preparation. In the first step, the RNA is fragmented, which can be done by physical, enzymatic or chemical means (Head et al., 2014). Specialized methods are used for the enrichment of a specific RNA molecule type in the sample. The ribo-depletion method removes ribosomal RNA to enrich messenger RNA, transfer RNA molecules and small non-coding RNA. Exome sequencing targets the mRNA sequence alone, using poly-A selection to remove any other forms of RNA (Hrdlickova et al., 2017).

The second step of the library preparation was the conversion of the RNA to cDNA. This conversion can be done in different ways, including by adding adapters, random priming, and priming with the oligo-dTs, and is followed by amplification for complexity (Hrdlickova et al., 2017). The final step is creating the library. The fragmented RNAs are prepared and are then loaded into a glass slide of flow cells. Each of these cells is coated with oligonucleotides. When the samples are allowed to hybridize to the oligonucleotides, they go through a bridge amplification process. Bridge

amplification is when the reverse and forward stands are created (Dündar et al., 2015). Sequencing at a single base resolution is finalized by the 'Sequencing by Synthesis' technology. The reversible chain terminations let repeated cyclical addition of bases and their subsequent florescence-based detection (Buermans & Den Dunnen, 2014).

The analysis of the RNA sequencing is still new and there is no standard protocol, but there are some recommended ways for data handling and analysis. The data from library preparation is received normally as FASTA or FASTQ files. Quality control is recommended for the raw unaligned reads to ensure the read qualities are ideal on the Phred scores. The Phred scores have a range of 10-60, which is the average base score at a position in the read; the higher the score, the better base calling, which is the process to select the bases to the cromatom peak, at that position. A score of 10, for example, means that there is 1 error base call in 10 base calls, corresponding to 10% error (Ewing et al., 1998). After the quality control, the data are aligned to the reference genome of interest.

There is a vast selection of aligners available to the user. They have two major subclasses: spliced or non-spliced. Spliced aligners can recognize intron gaps (Engstrom et al., 2013), while non-spliced aligners are used to align DNA sequencing runs' output and cannot identify the introns from the gaps in the alignment. Therefore, verification of the alignment is performed by the post-alignment quality control. Depending on the results, it might be necessary to perform read filtering or adapter trimming prior to performing expression quantification and differential expression measurements (Figure 6).

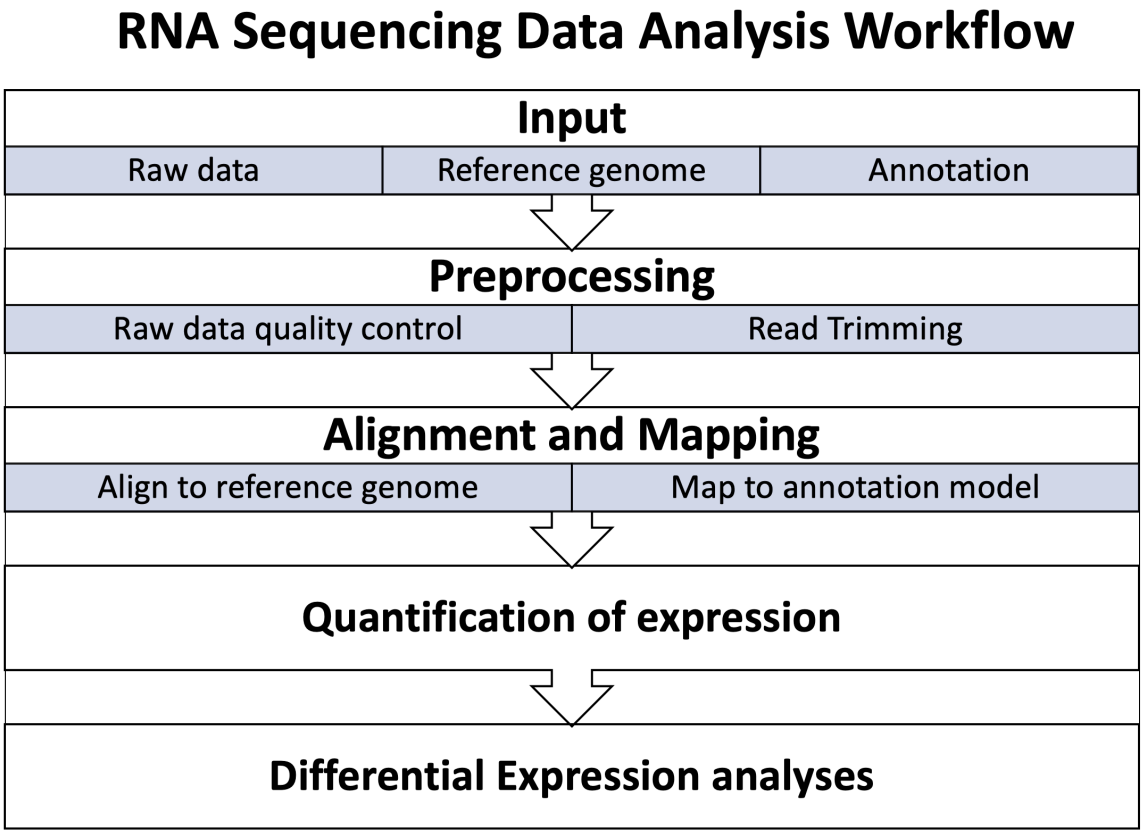

Figure 6. RNA Sequencing Data Analysis Workflow.

The goal of this study is to analyze what human genes of the five DNA repair pathways are conserved in elasmobranchs. With these five pathways and approximately 300 genes, a comparison was studied between the expression of the elasmobranch NER genes to the expression of the human NER genes. This project is the first to examine NER gene expression and the other four DNA repair pathways in elasmobranchs with RNA sequencing.

Materials and Methods

Study Species

This study included the nurse shark *Ginglymostoma cirratum* which has a longevity of *ca.* 25 years (Clark, 1963) and the yellow stingray *Urobatis jamaicensis* that has a shorter lifespan of *ca.* 7-8 years (Sulikowski, 1996). Yellow stingrays are found along sandy beaches and around coral reefs. They are carnivorous, feeding on small fish, crabs, polychaete worms, and other small crustaceans, such as shrimps. Yellow stingrays grow to a maximum of 66 cm in total length and a maximum disc width of approximately 35.5 cm (Compagno, 1999 & Sulikowski., 1996). Nurse sharks are found on continental and insular shelves. They are nocturnal, solitary and can often be found lying on the sand bottom. They feed on bottom-dwelling invertebrates like lobsters, shrimp, crabs, sea urchins, and squid, as well as demersal fishes (Matott et al., 2005). Nurse sharks reach a maximum total length of approximately 2.3 to 3 m (Rosa et al., 2006).

Specimen Collection

Specimens were collected in Broward County, Florida (USA). Nurse sharks (n=3) were brought onto the dock and physically restrained. Aseptic techniques were performed to take a 0.5 cm diameter and approximately circular sample of the dermis and underlying musculature. Because of the species' small size, yellow stingrays (n=3) were instead sampled in the laboratory after euthanasia using the same aseptic techniques. Samples were snap-frozen on dry ice immediately after collection. The wounds of the nurse sharks were swabbed with iodopovidone before the animal was released alive.

Figure 7. Harvesting the tissue samples of a nurse shark (left) and a yellow stingray (right).

Sampling collections occurred under Florida Fish and Wildlife Conservation Commission (FWC) permit SAL-17-1887-SRP to the co-PI, David Kerstetter (Halmos College of Natural Sciences and Oceanography). The procedures have been reviewed by the Nova Southeastern University Institutional Animal Care and Use Committee (IACUC) and occurred under approval 2017-DK1, also to the co-PI, David Kerstetter.

Foreskin fibroblast (FF) were prepared using the Latimer lab's process of cell culture protocol. The FF tissues were obtained from newborns after circumcision, then converted into primary explants following the process described by Latimer et al. (2003). The cells were placed on uncoated chamber where they were grown with MEM (Minimum Essential Medium Eagle) (REF #10-010-CV) containing 10% fetal calf serum. The cells were grown continuously for homogeneity in culture for up to 12 passages (a passage is the number of times the cells have been subculture).

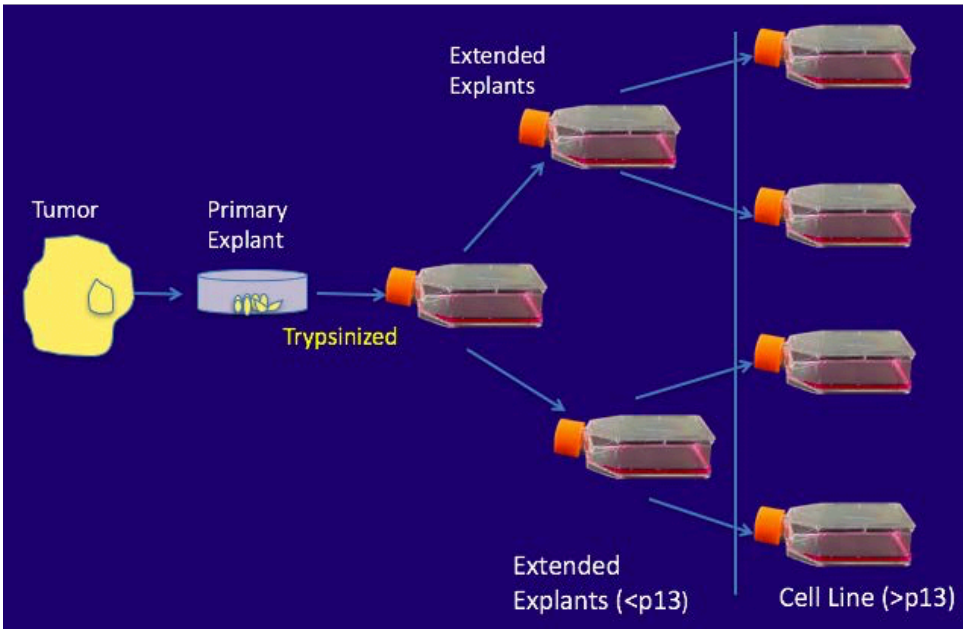

Figure 8. Summary schematic of the Latimer culture system and expansion. Tissues are minced then plated on a coated two-chamber slides in the MWRI medium and incubated in the incubator at 37°C and 5% CO_2. These cultures are called primary cultures, which are subsequently passaged into extended explants (< passage 13), then cell lines (> passage 13). Image from Homood (2017).

RNA preparation

The miRNeasy mini kit (Qiagen; Hilden, Germany) was used to isolate total RNA as per the manufacturer protocol. The samples were pulse homogenized on ice (i.e., while still frozen) in the presence of RNAse inhibitor for 2-4 minutes with a disposable tissue grinder (Omi International., Inc.; Kennesaw, GA, USA) that had been autoclaved and cleaned of RNAses previously with diethyl pyocarbonate (DEPC).

Analysis of RNA

RNA samples (1 ug of the total RNA per specimen) were sent to the NSU Genomics Core Facility at Nova Southeastern University for RNA sequencing. RNA samples were evaluated for quality and concentration using an Agilent Tapestation/Bioanalyzer (Agilent Technologies; Santa Clara, CA, USA). Samples were subjected to Illumina TruSeq Stranded Total RNA Library Preparation (whole transcriptome library generation, including cyt and mt rRNA removal) and sequenced on a 2x150 bp paired-end run using an Illumina 300 Cycles 400M flow cell (300-cycle, 400 million read; Illumina, Inc.; San Diego, CA, USA).

Data were delivered as Fastq files by the Genomics Core Facility, which were analyzed using Partek Flow software (Partek Inc.; Chesterfield, MO, USA). Pre-alignment QA/QC was performed, after which the raw reads for nurse shark and yellow stingray samples were aligned to the elephant shark reference genome, and the human samples to the human reference genome (hg38), using the aligner Burrows-Wheeler Aligner (BWA). An average of 197 million paired-end reads per sample (or 99 million reads/clusters per sample) was obtained. Upon confirming optimum alignment by post-alignment QA/QC, reads were quantified by the elephant shark's annotation model from National Center for Biotechnology Information (NCBI) using Partek's E/M algorithm that uses RPKM scaling to give gene and transcript counts. The genes were filtered by each pathway list and downloaded to the .txt file (Figure 5).

The repair expression was expressed as a percentile of human foreskin fibroblasts. Pairwise Student's t-tests (significant at $p<0.05$) were performed for each gene comparing the read counts for nurse shark and yellow stingray samples, respectively, to the human foreskin fibroblasts.

Homology

The reference genome used in this study was derived from the elephant shark *Callorhinchus milii*, also called the Australian ghostshark. While a chimaera (Subclass Holocephali) and thus technically not a true shark, the elephant shark is still in Class Chondrichthyes and remains the most closely related species with a known reference genome to the nurse shark and the yellow stingray (both Subclass Elasmobranchii). The elephant shark provides a critical reference to understand the evolution of the vertebrate genome evolution, which provides the whole-genome sequence and comparative analysis. Gene sequences from the elephant shark were obtained from NCBI for each NER gene in the species. Gene sequences were checked for homology with the human genome (hg38) by the Basic Local Alignment Tool (BLAST) in the NCBI website.

Results

NER

Humans have twenty canonical genes that are necessary for DNA repair. Fourteen out of twenty of these genes (70%) are present in the elephant shark genome represented in Figures 7, 8, 9 and Table 1. These genes were analyzed in both the nurse shark and the yellow stingray. It was assumed that there was insufficient homology in the remaining six NER genes to allow for analysis.

Homology was assessed in the fourteen genes found in the elephant shark genome that compared with the human genome. The homology ranged from 67% to 89%: TFIIHp34 (GTF2H3)=67%, RPA3=72%, XPF (ERCC4)=73%, RPA1=75%, XPC=76%, CCNH=77%, DDB1=80%, XPA=81%, XPB (ERCC3)=81%,TFIIHp44 (GTF2H2)=82%, CSB (ERCC6)=83%,RPA2=86%, CSA (ERCC8)=87% and CDK7=89%. The homology observed between elephant shark and human averaged 79% overall in the NER pathway in the 14 genes.

Expression of the NER genes present in nurse shark and yellow stingray were compared to those found in humans. In nurse shark, eight out of the fourteen genes were significantly different (Figure 10). Five out of the eight genes had lower expression in nurse sharks than humans, and all five of these genes are involved in global genomic

repair: CCNH ($p = 0.029$), DDB1 ($p = 9x10^{-4}$), TFIIH ($p = 0.016$), RPA3 ($p = 0.002$), and XPC ($p = 0.001$). In contrast, nurse shark gene expression is significantly higher in three genes, two of which are involved in transcription coupled repair: CSB ($p = 0.015$), CSA ($p = 0.007$) and XPF ($p = 0.0127$). In the yellow stingray, seven genes were significantly higher in humans than in yellow stingray: CDK7 ($p = 5x10^{-4}$), DDB1 ($p = 8x10^{-4}$), TFIIH ($p = 0.012$), RPA1 ($p = 0.043$), RPA3 ($p = 0.001$), XPA ($p = 0.015$), and XPC ($p = 0.001$) (Figure 10).

Between nurse shark and yellow stingray, seven out of the fourteen NER genes showed significant differences. Six out of the seven genes were significantly higher in nurse sharks than in yellow stingrays. CCNH (p = 6x10-4), XPF ($p = 0.016$), CBS ($p = 0.035$), TFIIH (p = 2x10-4), RPA1 ($p = 0.009$), RPA3 ($p = 0.001$), and XPA ($p = 0.005$). One gene was significantly lower in nurse sharks compared to yellow stingrays, CCNH (p = 6x10-4) (Figure 11).

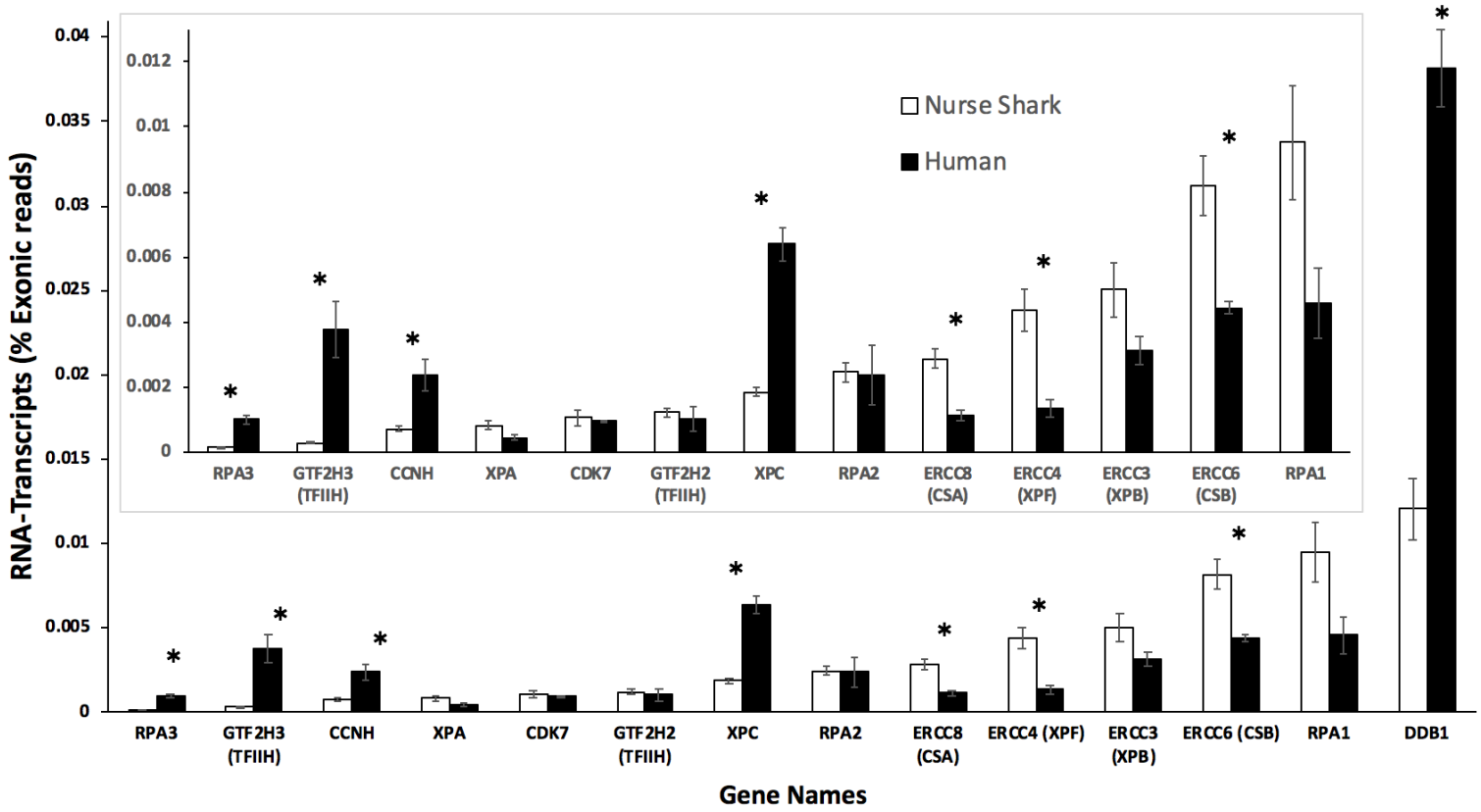

Figure 9. Nucleotide Excision Repair Gene Expression Nurse Shark vs. Human. Each nurse shark and human samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$ level.). Eight out of the fourteen genes were significantly different. Five out of the eight genes had lower expression in sharks than humans; CCNH ($p = 0.029$), DDB1 ($p = 9x10^{-4}$), TFIIH ($p = 0.016$), RPA3 ($p = 0.002$), and XPC ($p = 0.001$). Nurse shark genes expression is significantly higher in three genes; CSB ($p = 0.015$), CSA ($p = 0.007$) and XPF ($p = 0.0127$).

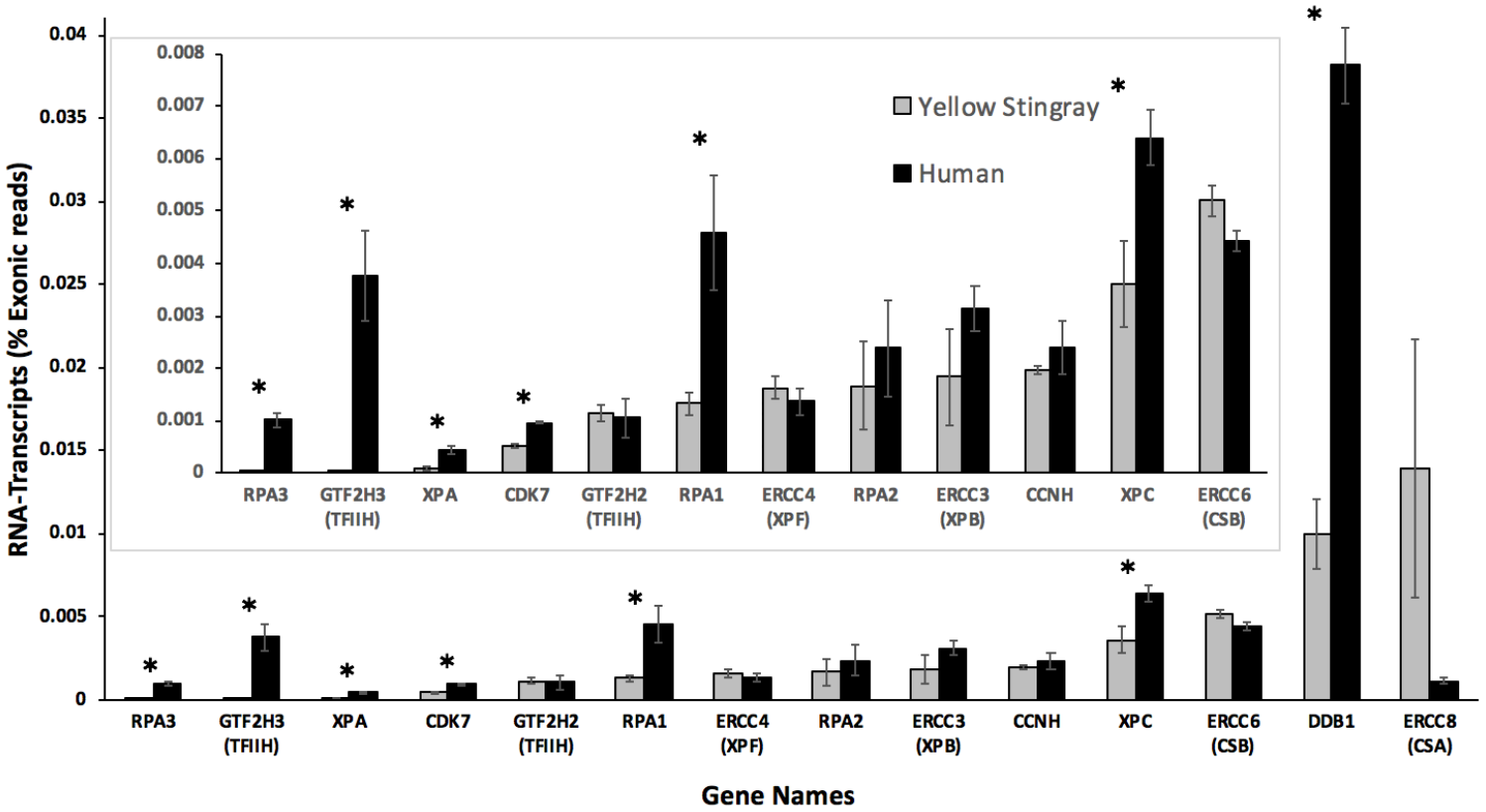

Figure 10. Nucleotide Excision Repair Gene Expression Yellow stingray vs. Human. Each yellow stingray and human samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$ level.). Seven out of the fourteen genes were significantly higher in human than yellow stingray; CDK7 ($p = 5x10\text{-}4$), DDB1 ($p = 8x10\text{-}4$), TFIIH ($p = 0.012$), RPA1 ($p = 0.043$), RPA3 ($p = 0.001$), XPA ($p = 0.015$), and XPC ($p = 0.001$).

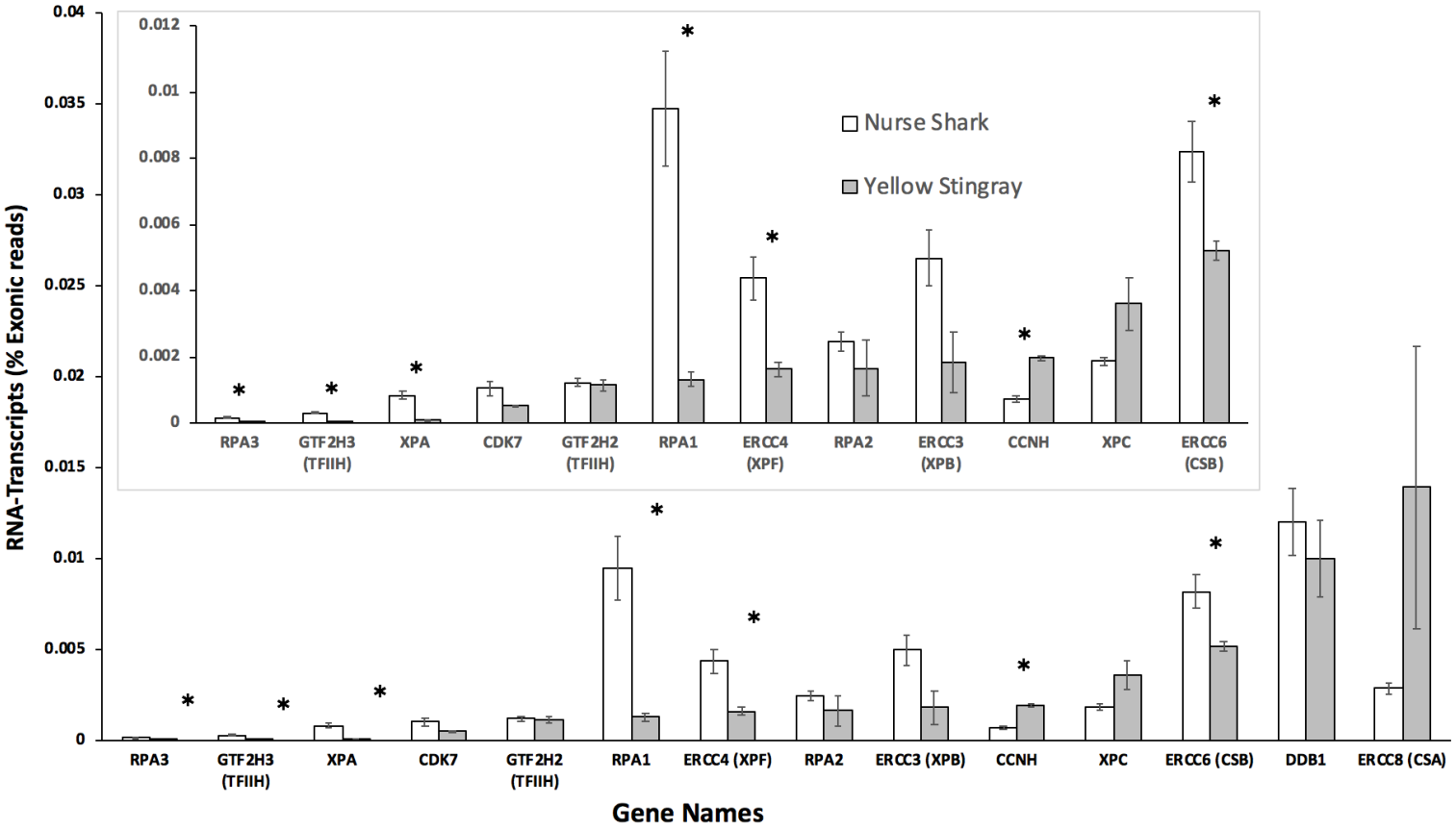

Figure 11 .Nucleotide Excision Repair Gene Expression Yellow stingray vs. Nurse Shark. Each nurse shark and yellow stingray samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$ level.). Seven out fourteen genes are significantly different. Six out of these genes are significantly higher in nurse shark compared to yellow stingray; CCNH (p =6 x10-4), XPF (p = 0.016), CBS (p = 0.035), TFIIH (p = $2x10^{-4}$), RPA1p = 0.009), RPA3 (p = 0.001), and XPA (p =0.005). One gene was significantly higher in yellow stingray compared to nurse shark; CCNH (P = $6x10^{-4}$)

Table 6. Canonical NER genes, function, and their %homology between elephant shark and human. Genes in red are not present in the elephant shark.

GENES	FUNCTION	HOMOLOGY
CDK7	CTD kinase	89
CSA (ERCC8)	5' Endonuclease	87
RPAp32 (RPA2)	Repair initiation	86
CSB (ERCC6)	5'-3 Helicase	83
TFIIHp44 (GTF2H2)	DNA unwinding	82
XPA	Initiation of repair	81
XPB (ERCC3)	3'-5' Helicase	81
DDB1	Recognition (Global Genome-NER)	80
CCNH	DNA unwinding	77
XPC	Recognition (Global Genome-NER)	76
RPAp70 (RPA1)	Repair initiation	75
XPF (ERCC4)	5' Endonuclease	73
RPAp14 (RPA3)	Repair initiation	72
TFIIHp34 (GTF2H3)	DNA unwinding	67
XPD (ERCC2)	5'-3' Helicase	-
XPE	Recognition	-
XPG (ERCC5)	3' Endonuclease	-
ERCC1	5' Endonuclease	-
hHRAD23B	Recognition (Global Genome-NER)	-
TFIIHp52 (GTF2H4)	DNA unwinding	-

BER

Out of the twenty-eight important genes in the human BER pathway, eighteen (70 %) were found in the elephant shark genome.

Expressions of the BER genes present in nurse shark and yellow stingray were each compared to human. Eight out the eighteen genes were significantly different in nurse shark versus human: POLB ($p = 0.043$) and POLE ($p = 0.035$) (Figure 12). Out the eight genes, two were significantly higher in the nurse shark than human; the other six genes were significantly lower in nurse shark than human: POLE4 ($p = 0.0005$), OGG1 ($p = 0.0002$), UNG ($p = 0.0001$), SMUG1 ($p = 0.0001$), MPG ($p = 0.009$) and POLD2 ($p = 0.0005$). Six out the eighteen genes were significant in the yellow stingray versus human (Figure 13). Five out the six genes were significantly lower in yellow stingray than in human: MPG ($p = 0.007$), OGG1 ($p = 0.0001$), POLD2 ($p = 0.0001$), POLE4 ($p = 0.001$) and UNG (0.0001). Only one gene was significantly higher in yellow stingray than human: NTHL1 ($p = 0.040$).

The comparation of the expression of the BER genes present in nurse shark and yellow stingray found eleven out of eighteen genes significantly different (Figure 14). Eight gene expressions of the eleven were significantly higher in nurse shark compared to the yellow stingray: MPG ($p = 0.003$), OGG1 ($p = 0.01$), POLD3 ($p = 0.0004$), POLE ($p = 0.025$) PARP1 ($p = 0.027$), POLB ($p = 0.030$), POLE3 ($p = 0.012$) and POLD2 ($p = 0.00003$). Nurse shark had three significantly lower gene expressions compared to the yellow stingray out the eleven genes: NTHL1 ($p = 0.026$), POLE4 ($p = 0.046$) and SMUG1 ($p = 0.049$).

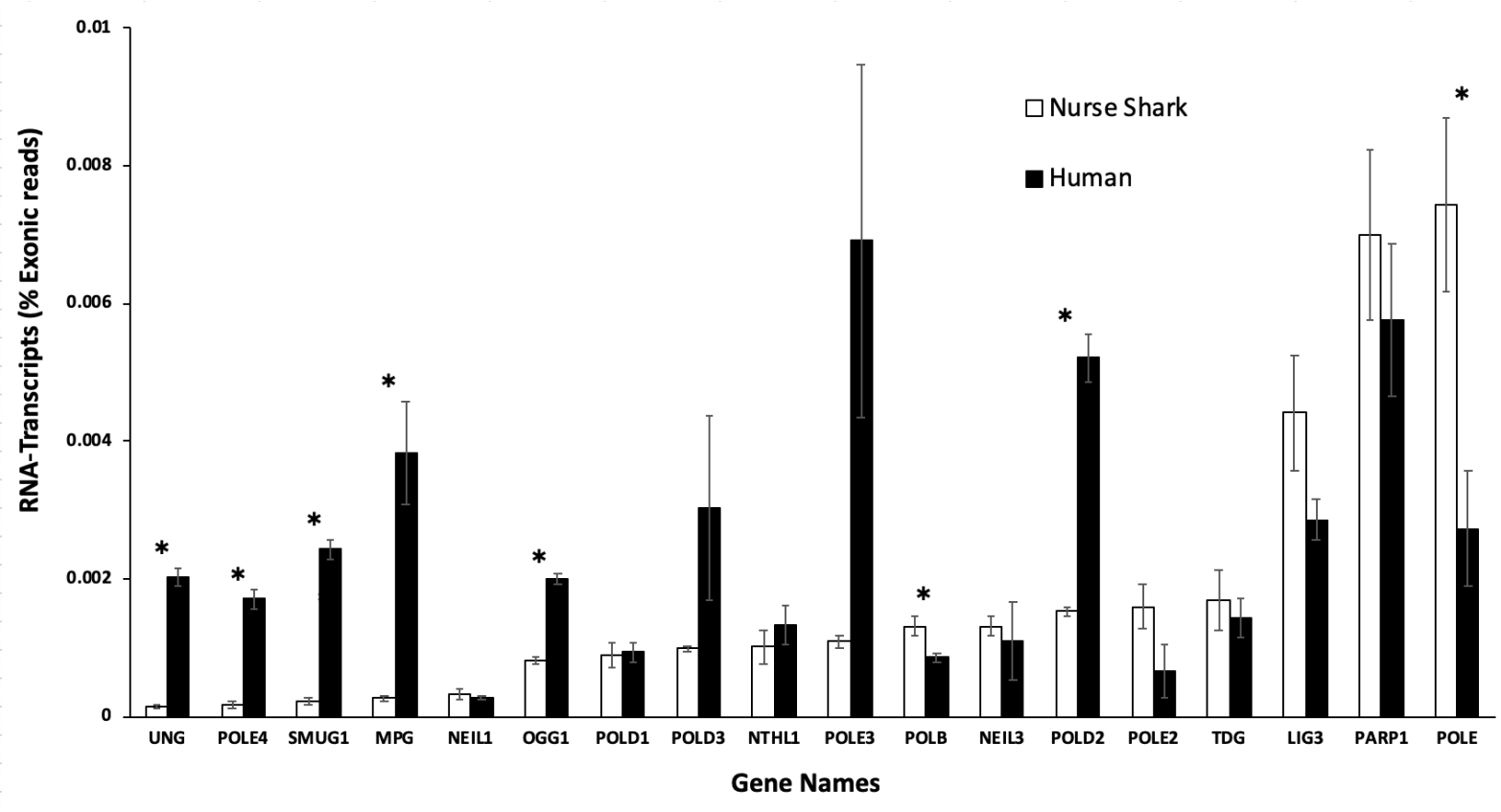

Figure 12. Base Excision Repair Gene Expression Nurse Shark vs. Human. Each nurse shark and human samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$ level.) Eight out of the eighteen gene expressions are significantly different. Humans showed six significantly higher gene expressions compared to the nurse shark: POLE4 ($p = 0.0005$), OGG1 ($p = 0.0002$), UNG ($p = 0.0001$), SMUG1 ($p = 0.0001$), MPG ($p = 0.009$) and POLD2 ($p = 0.0005$), while two gene expressions were significantly higher in nurse shark: POLB ($p = 0.043$) and POLE ($p = 0.035$).

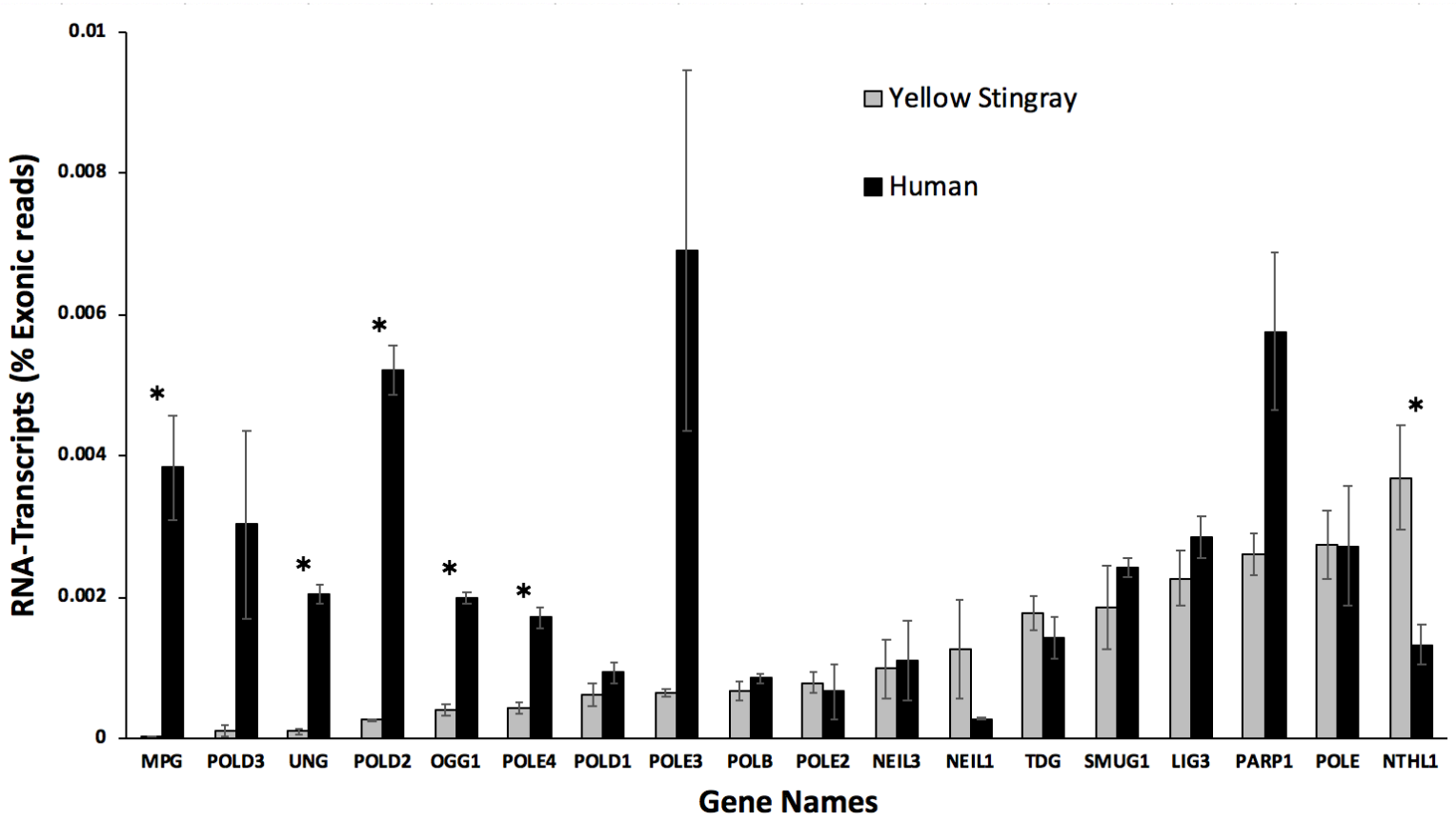

Figure 13. Base Excision Repair Gene Expression Yellow stingray vs. Human. Each yellow stingray and human samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$ level.) Six out of the eighteen gene expressions are significantly different. Five gene expressions are significantly higher in human: MPG ($p = 0.007$), OGG1($p = 0.0001$), POLD2 ($p = 0.0001$), POLE4 ($p = 0.001$), and UNG (0.0001), while only one expression was significant higher in yellow stingray: NTHL1 ($p = 0.040$).

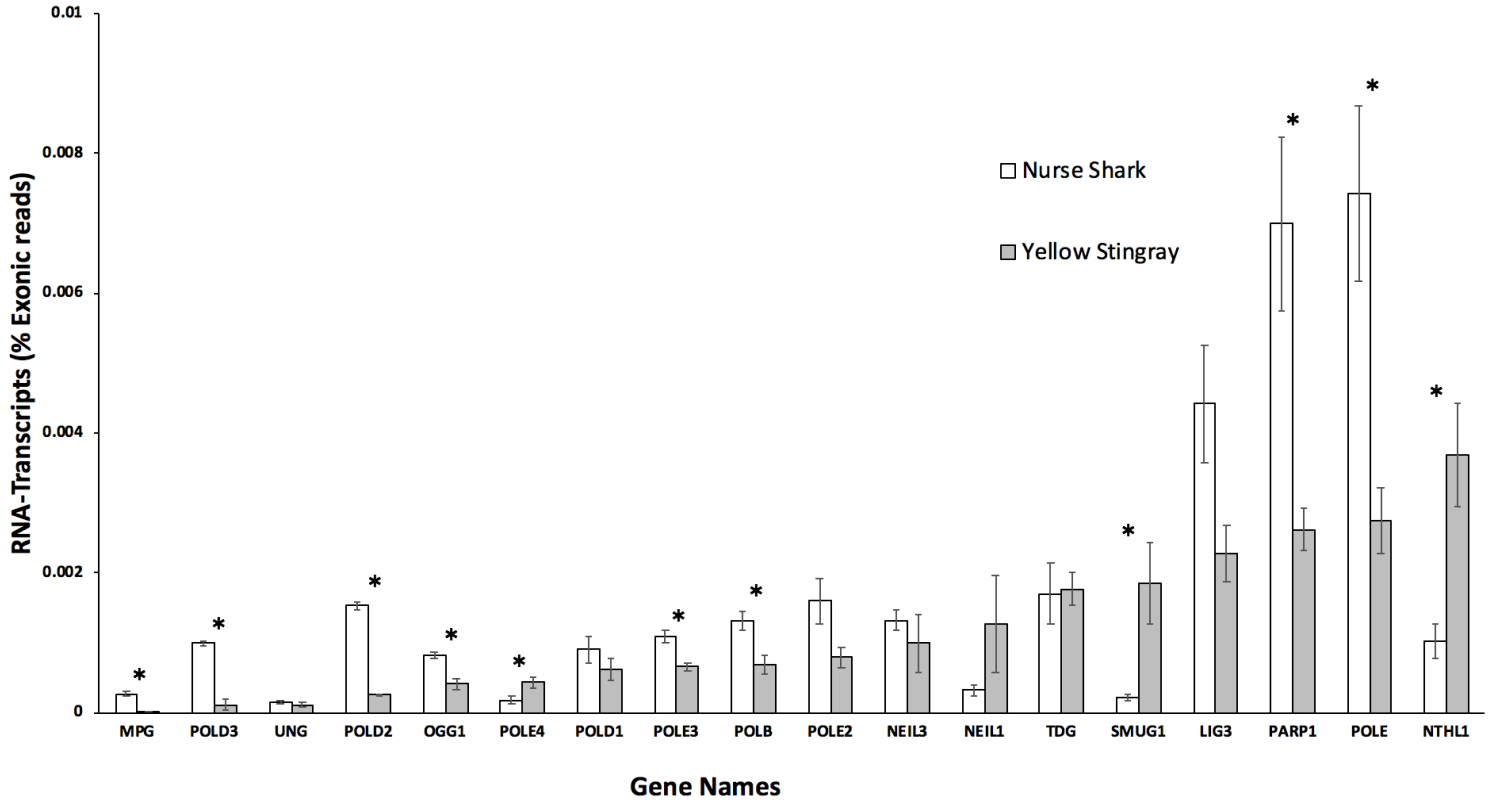

Figure 14. Base Excision Repair Gene Expression Yellow stingray vs. Nurse Shark. Each nurse shark and yellow stingray samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$ level.) Eleven out of eighteen gene expressions are significantly different. Eight of those genes were significantly higher in nurse shark compared with yellow stingray: MPG ($p = 0.003$), OGG1 ($p = 0.01$), POLD3 ($p = 0.0004$), POLE ($p = 0.025$), PARP1 ($p = 0.027$), POLB ($p = 0.030$), POLE3 ($p = 0.012$), and POLD2 ($p = 0.00003$), while three gene expressions are significantly higher in yellow stingray: NTHL1 ($p = 0.026$), POLE4 ($p = 0.046$), and SMUG1 ($p = 0.049$).

MMR

Out of the thirty-seven important genes in the human MMR pathway, we found twenty-three in the elephant shark genome (66%).

Expression of the MMR genes present in the nurse shark and the yellow stingray were compared to the human. In the nurse shark, eight out of the twenty-three genes were significantly higher than in human: MRE11 ($p = 0.009$), MSH3 ($p = 0.019$), MSH4 ($p = 0.003$), MUTYH ($p = 0.014$), PMS1 ($p = 0.013$), PRKCZ ($p = 0.003$), TP73 ($p = 0.006$), and YBX1 ($p = 0.003$) (Figure 15).

In the yellow stingray, ten out of the twenty-three gene expressions were significantly different. Four gene expressions were significantly lower than human: MBD4 ($p = 0.04$), MLH1 ($p = 0.047$), RCCD1 ($p = 0.0009$), and RPA1 ($p = 0.043$). In contrast, yellow stingray gene expression is significantly higher in six genes as compared to human: MSH3 ($p = 0.049$), MSH4 ($p = 6.5 \times 10^{-6}$), PMS2 ($p = 0.0083$), PRKCZ ($p = 0.016$), TP73 ($p = 0.039$), and YBX1 ($p = 0.001$) (Figure 16).

Between the nurse shark and the yellow stingray, eight out of twenty-three MMR gene expressions showed significant differences (Figure 17). Only two out of the eight gene expressions were significantly lower in the stingrays versus the nurse shark: MSH4 ($p = 0.0002$) and PMS2 ($p = 0.014$). In the nurse shark, gene expressions were significantly higher in six out of the eight genes compared to the yellow stingray: BLM ($p = 0.005$), MLH1 ($p = 0.049$), MRE11 ($p = 0.006$), POLD3 ($p = 0.0004$), RCCD1 ($p = 0.006$), and RPA1 ($p = 0.009$).

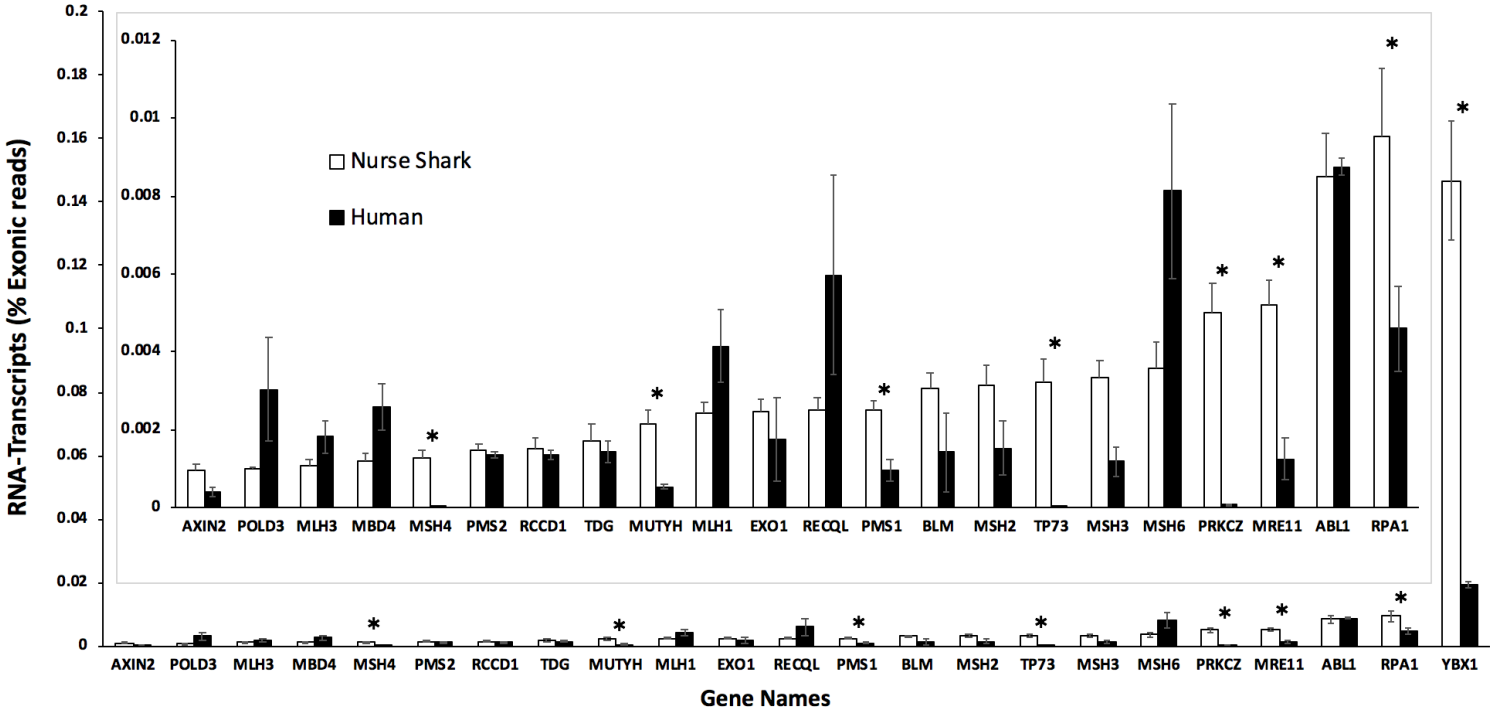

Figure 15. DNA Mismatch Repair Gene Expression Nurse Shark vs. Human. The nurse shark and human samples have three biological replicates each. (Asterisk (*) indicates significance at $p < 0.05$ level.) Eight out the twenty-three gene expressions in the MMR pathway are significantly different. All the eight gene expressions are significantly higher in the nurse shark genes: MRE11 ($p = 0.009$), MSH3 ($p = 0.019$), MSH4 ($p = 0.003$), MUTYH ($p = 0.014$), PMS1 ($p = 0.013$), PRKCZ ($p = 0.003$), TP73 ($p = 0.006$), and YBX1 ($p = 0.003$). The inserted graph in the original picture represent the genes without the outlier gene results (YBX1).

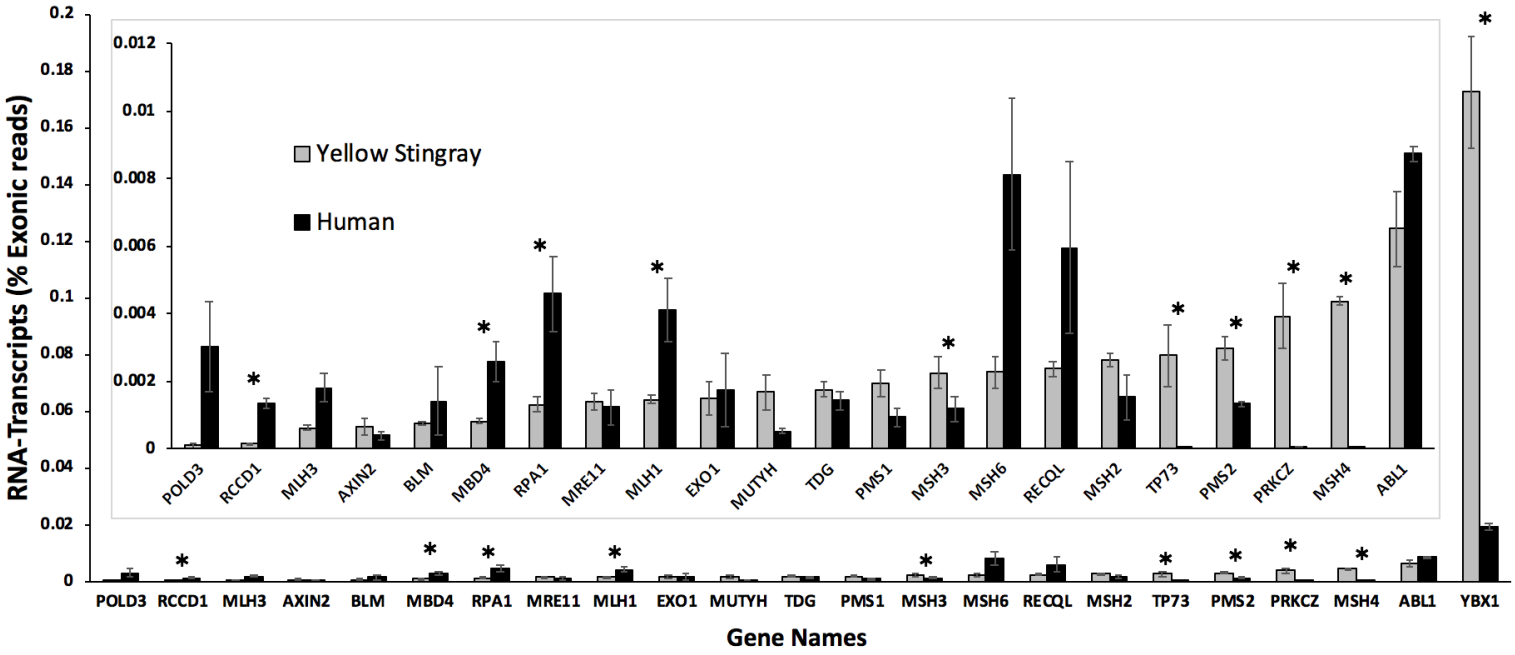

Figure 16. DNA Mismatch Repair Gene Expression Yellow stingray vs. Human. The human and yellow stingray samples have three biological replicates each. (Asterisk (*) indicates significance at $p < 0.05$ level.) Ten out of the twenty-three gene expressions are significantly different in the MMR pathway between the yellow stingray and the human samples. Six of those gene expressions were significantly higher in yellow stingray: MSH3 ($p = 0.049$), MSH4 ($p = 6.5 \times 10^{-6}$), PMS2 ($p = 0.0083$), PRKCZ ($p = 0.016$), TP73 ($p = 0.039$), and YBX1 ($p = 0.001$), while the human samples have four significantly higher gene expressions: MBD4 ($p = 0.04$), MLH1 ($p = 0.047$), RCCD1 ($p = 0.0009$), and RPA1 ($p = 0.043$). The inserted graph in the original picture represents the genes without the outlier gene results (YBX1).

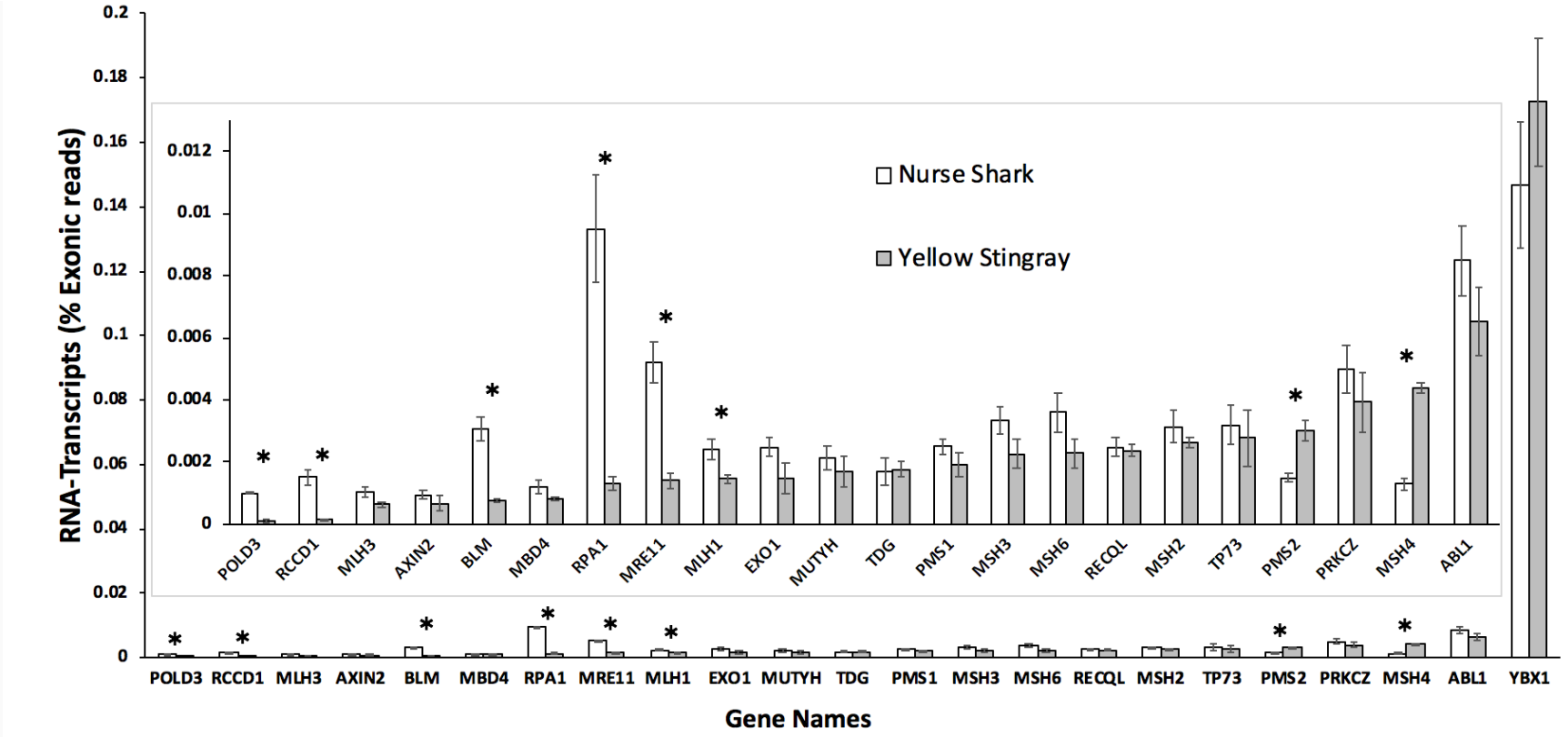

Figure 17. DNA Mismatch Repair Gene Expression Yellow stingray vs. Nurse Shark. Each nurse shark and yellow stingray samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$ level.) Eight out of the twenty-three gene expressions were significantly different in the MMR pathway in the comparison of nurse shark and yellow stingray. Six of those gene expressions were significantly higher in nurse shark vs yellow stingray: BLM ($p = 0.005$), MLH1 ($p = 0.049$), MRE11 ($p = 0.006$), POLD3 ($p = 0.0004$), RCCD1($p = 0.006$), and RPA1 ($p = 0.009$), while significantly lower in two genes: MSH4 ($p = 0.0002$) and PMS2 ($p = 0.014$). The inserted graph in the original picture represent the genes without the outlier gene results (YBX1).

HR

Out of the thirty-two genes known in the human HR pathway, we found twenty-six in the elephant shark genome (84%).

Expression of the HR genes present in nurse shark and yellow stingray were compared to human. In nurse shark, ten out the twenty-six gene expressions were significantly different in nurse shark versus human (Figure 18). Out the ten gene expressions, eight were significantly higher in the nurse shark: BRCA2 ($p = 0.038$), FSBP ($p = 0.004$), MRE11A ($p = 0.009$), RAD51B ($p = 0.043$), RAD54B ($p = 0.002$), RAD54L ($p = 0.004$), UBE2N ($p = 0.024$), and XRCC3 ($p = 0.014$). Nurse shark gene expressions were significantly lower in two out the ten significant genes compared to human: POLD2 ($p = 0.0005$) and RAD51D ($p = 0.044$).

In yellow stingray, five out the twenty-six gene expressions were significantly different compared to human (Figure 19). Three gene expressions were significant lower in yellow stingray than in human: POLD2 ($p = 0.0001$), RAD51D ($p = 0.004$), and RPA1 ($p = 0.043$). Two gene expressions were significantly higher in yellow stingray than human: RAD54B ($p = 0.046$) and SHFM1 ($p = 0.012$).

Between nurse shark and yellow stingray, eleven out of twenty-six HR gene expressions showed significant differences (Figure 20). Nine gene expressions of those were significantly higher in nurse shark compared to the yellow stingray: BLM ($p = 0.005$), MRE11A ($p = 0.006$), POLD2 ($p = 2.83 \times 10^{-5}$), POLD3 ($p = 0.0004$), RAD51C (0.004), RAD51D ($p = 0.011$), RAD54L ($p = 0.039$), RPA1 ($p = 0.009$), and ERCC3 ($p = 0.011$). Nurse shark had two gene expressions significantly lower than in yellow stingray: SHFM1($p = 0.011$) and XRCC2 ($p = 6.8 \times 10^{-5}$).

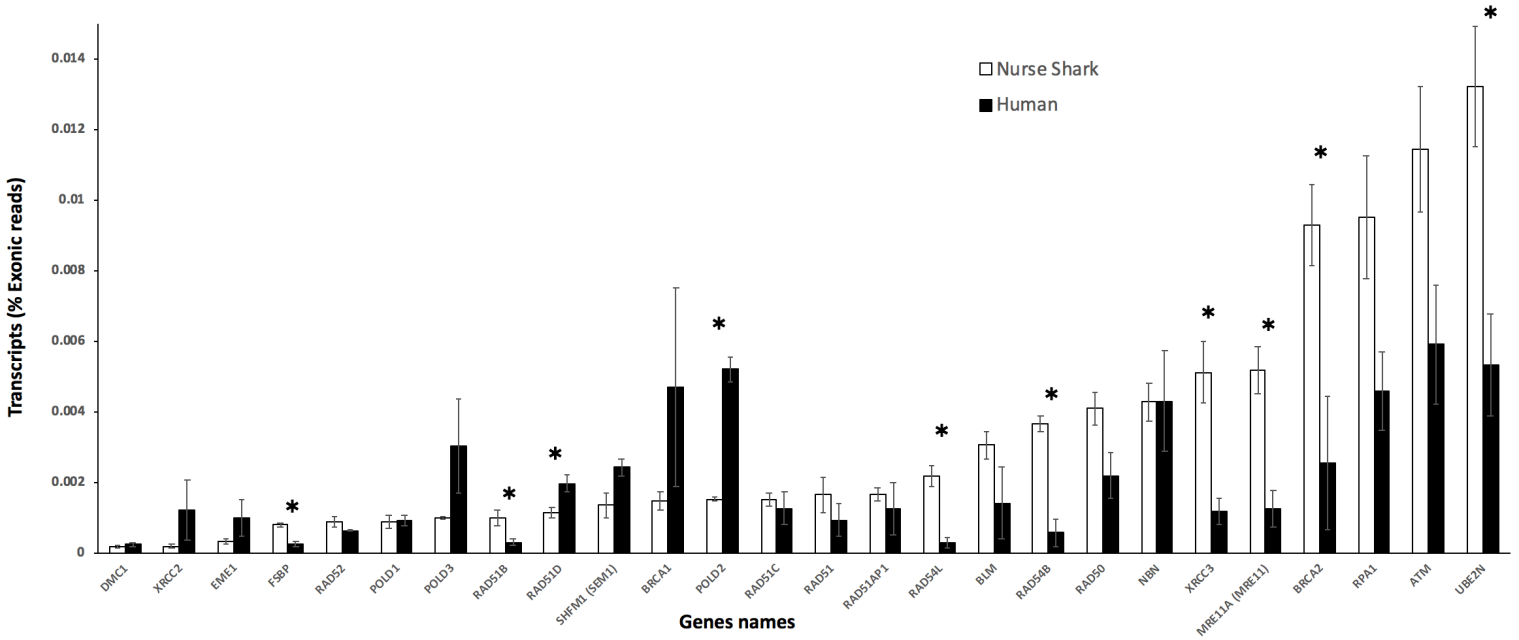

Figure 18. Homologous Recombination Gene Expression Nurse Shark vs. Human. Nurse shark and human samples have three biological replicates each. (Asterisk (*) indicates significance at $p < 0.05$ level.) Ten out the twenty-six gene expressions were significantly different in nurse shark versus human. Out the ten genes, eight gene expressions were significantly higher in the nurse shark: BRCA2 ($p = 0.038$), FSBP ($p = 0.004$), MRE11A ($p = 0.009$), RAD51B ($p = 0.043$), RAD54B ($p = 0.002$), RAD54L ($p = 0.004$), UBE2N ($p = 0.024$), and XRCC3 ($p = 0.014$), while human gene expressions were significantly higher in two out the ten genes: POLD2 ($p = 0.0005$) and RAD51D ($p = 0.044$).

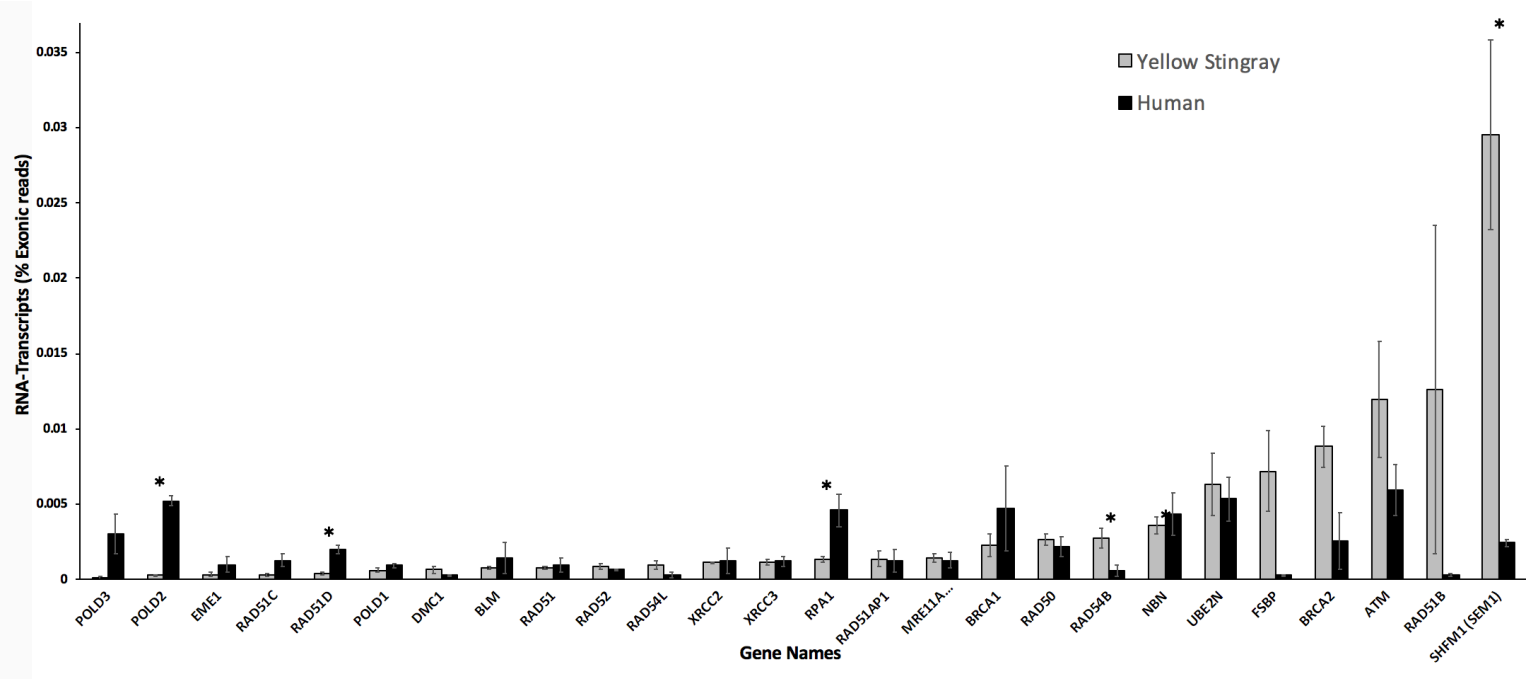

Figure 19. Homologous Recombination Gene Expression Yellow stingray vs. Human. Each human and yellow stingray samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$.). Gene expressions for five out the twenty-six genes were significantly different. Three out of those gene expressions were significant higher in human than in rays: POLD2 ($p = 0.0001$), RAD51D ($p = 0.004$), and RPA1 ($p = 0.043$), while two genes were significantly higher in yellow stingray: RAD54B ($p = 0.046$) and SHFM1 ($p = 0.012$).

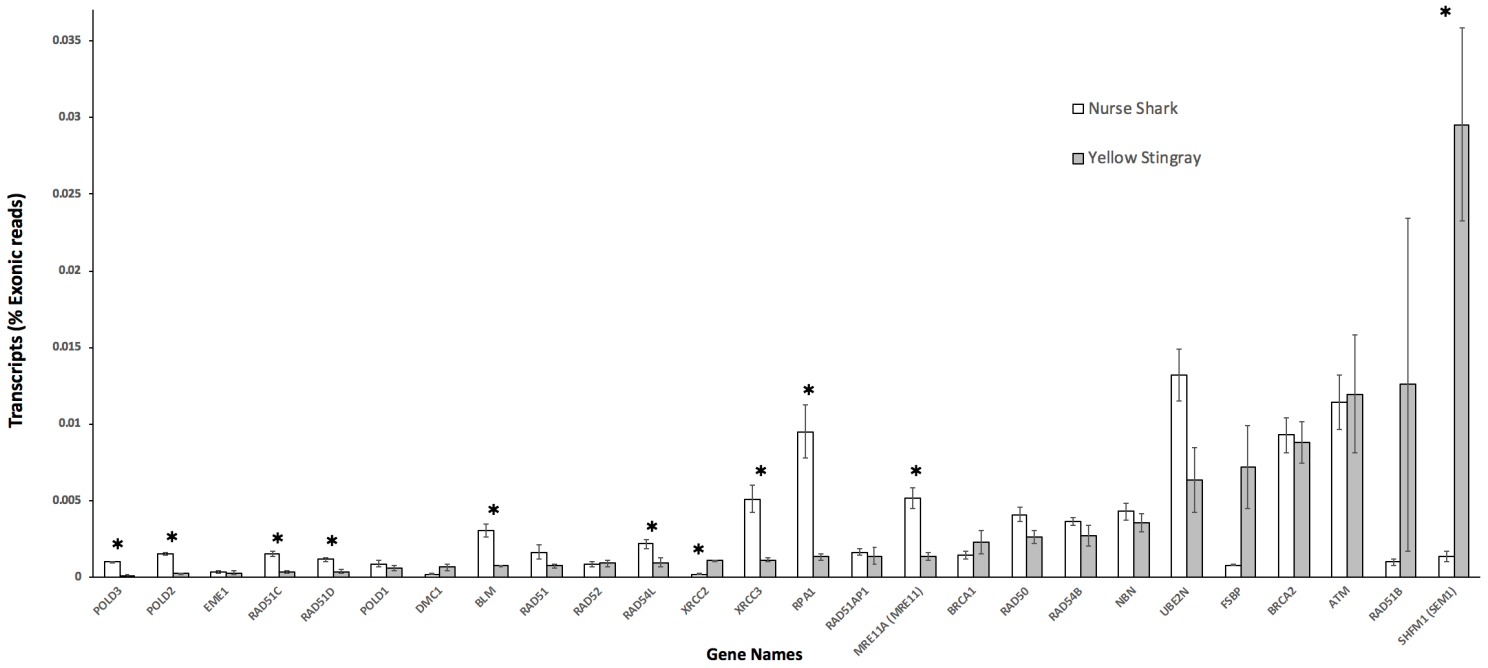

Figure 20. Homologous Recombination Gene Expression Yellow stingray vs. Nurse Shark. Nurse shark and yellow stingray samples have three biological replicates each. (Asterisk (*) indicates significance at $p < 0.05$.) Eleven out of twenty-six genes are significantly different for gene expressions. Nine genes of those are significantly higher in nurse shark compared to the yellow stingray: BLM ($p = 0.005$), MRE11A ($p = 0.006$), POLD2 ($p = 2.83 \times 10^{-5}$), POLD3 ($p = 0.0004$), RAD51C (0.004), RAD51D ($p = 0.011$), RAD54L ($p = 0.039$), RPA1 (0.009), and ERCC3 (0.011), while yellow stingray gene expressions were significantly higher for two genes: SHFM1 ($p = 0.011$) and XRCC2 ($p = 6.8 \times 10^{-5}$).

NHEJ

Out of the nineteen important genes in the human NHEJ pathway, we found fifteen genes in the elephant shark genome (78%).

Expression of the NHEJ genes present in nurse shark and yellow stingray were compared to human. In nurse shark, gene expressions for five out of the fifteen genes were significantly higher than in human: APLF ($p = 0.0004$), LIG4 ($p = 0.012$), MRE11A ($p = 0.009$), SLC23A3 ($p = 0.003$), and XRCC6BP1 ($p = 0.004$). In contrast, gene expressions for nurse shark genes were significantly lower in three out the fifteen genes: NHEJ1 ($p = 0.0004$), XRCC5 ($p = 0.016$), and XRCC6 ($p = 4.19 \times 10^{-5}$) (Figure 21).

In yellow stingray, gene expressions for eight out of the fifteen genes were significantly different than in human. In yellow stingray, gene expressions for four out the eight genes were significantly higher in compared with human: APLF ($p = 0.019$), LIG4 ($p = 0.009$), SLC23A3 ($p = 0.007$), and XRCC6BP1 ($p = 0.003$). However, gene expressions for the yellow stingray samples were significant lower in four out the eight genes: NHEJ1 ($p = 0.0001$), POLL ($p = 0.001$), XRCC5 ($p = 0.014$), and XRCC6 ($p = 9.43 \times 10^{-5}$) (Figure 22).

Between nurse shark and yellow stingray, gene expressions for six out of the fifteen genes were significantly different (Figure 23). Nurse shark had six genes that were significantly higher than in the yellow stingray: APLF ($p = 0.009$), DCLRE1C ($p = 0.015$), MRE11A ($p = 0.006$), POLL ($p = 0.005$), SLC23A3 ($p = 0.007$), and XRCC5 ($p = 0.008$).

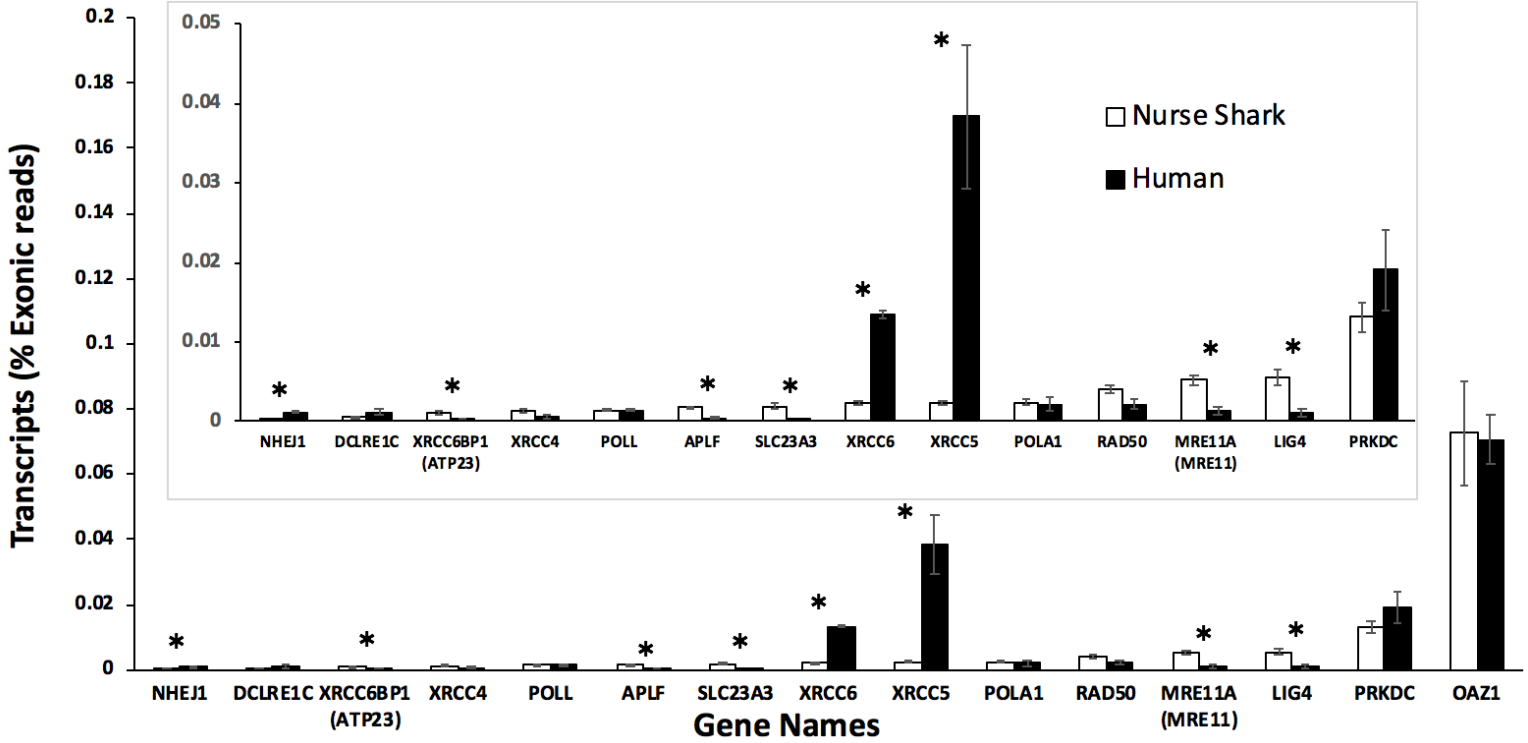

Figure 21. Non-Homologous End Joining Gene Expression Nurse Shark vs. Human. Each nurse shark and human samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$.) Gene expressions for eight out of the fifteen genes are significantly different. Five out of the fifteen genes are significantly higher in nurse shark genes: APLF ($p = 0.0004$), LIG4 ($p = 0.012$), MRE11A ($p = 0.009$), SLC23A3 ($p = 0.003$), and XRCC6BP1 ($p = 0.004$), while gene expressions for three genes are significantly higher in human: NHEJ1 ($p = 0.0004$), XRCC5 ($p = 0.016$), and XRCC6 ($p = 4.19 \times 10^{-5}$). The inserted graph in the original picture represent the genes without the higher exonic reads that are obstructing the significant genes.

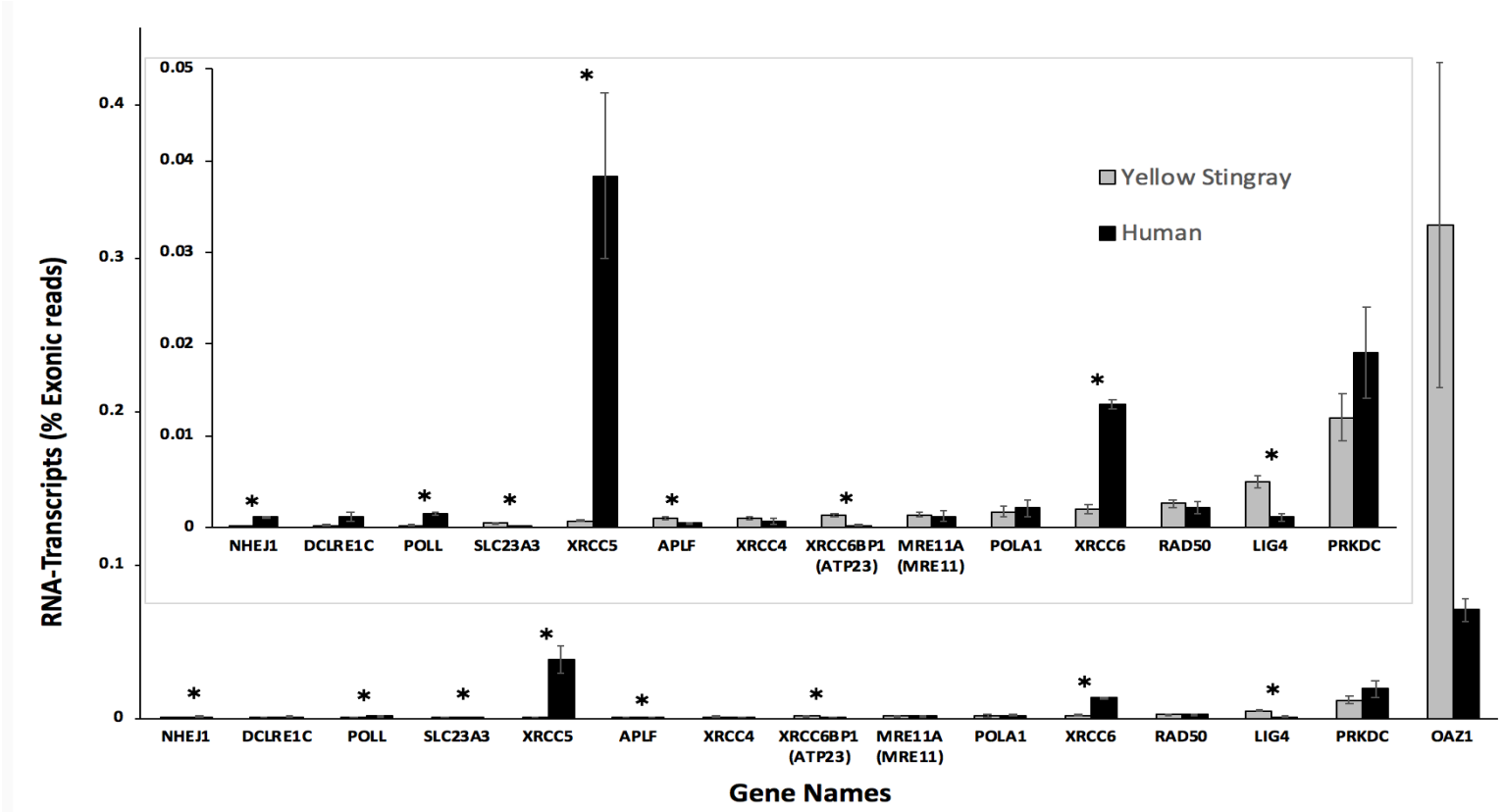

Figure 22. Non-Homologous End Joining Gene Expression Yellow stingray vs. Human. Each human and yellow stingray samples have three biological replicates. (Asterisk (*) indicates significance at $p < 0.05$.) Gene expressions for eight out of the fifteen genes are significantly different. The human samples were significant higher in four out of the eight genes: NHEJ1 ($p = 0.0001$), POLL ($p = 0.001$), XRCC5 ($p = 0.014$), and XRCC6 ($p = 9.43 \times 10^{-5}$), while the yellow stingray gene expressions were significantly higher in four out the eight genes: APLF ($p = 0.019$), LIG4 ($p = 0.009$), SLC23A3 ($p = 0.007$), and XRCC6BP1 ($p = 0.003$). The inserted graph in the original picture represent the genes without the outlier gene (OAZ1).

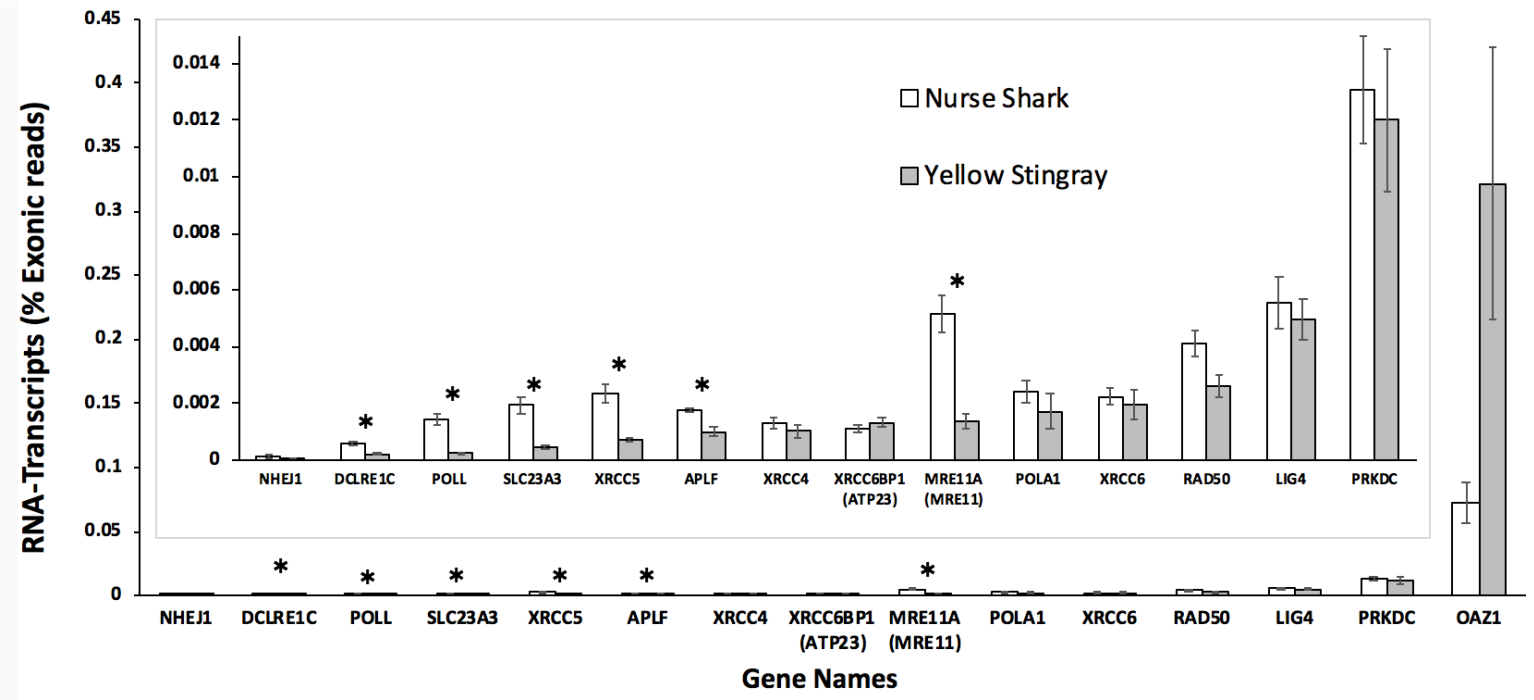

Figure 23. Non-Homologous End Joining Gene Expression Yellow stingray vs. Nurse Shark. Nurse shark and yellow stingray samples have three biological replicates each. (Asterisk (*) indicates significance at $p < 0.05$.) Gene expressions for six out of the fifteen genes were significantly different, all significantly higher in nurse shark: APLF ($p = 0.009$), DCLRE1C ($p = 0.015$), MRE11A ($p = 0.006$), POLL ($p = 0.005$), SLC23A3 ($p = 0.007$), and XRCC5 ($p = 0.008$). The inserted graph in the original picture represent the genes without the outlier gene (OAZ1).

Table 7. Gene expression results summary for genes used in the five DNA repair pathways.

	Significantly higher in nurse shark	Significantly higher in yellow stingray	Significantly higher in human
NER Genes (14 total)			
Shark vs Human	3		7
Ray vs Human			7
Shark vs Ray	6		1
BER Genes (18 total)			
Shark vs Human	2		6
Ray vs Human		1	5
Shark vs Ray	8		3
MMR Genes (23 total)			
Shark vs Human	8		
Ray vs Human		6	4
Shark vs Ray	6		2
HR Genes (26 total)			
Shark vs Human	8		2
Ray vs Human		2	3
Shark vs Ray	9		2
NHEJ Genes (15 total)			
Shark vs Human	5		3
Ray vs Human		4	4
Shark vs Ray	6		

Discussion

More than half of the genes were found to be conserved between the human and the elephant shark for all five repair pathways investigated. The lowest is MMR pathway with only 23 out 37 of the genes found conserved. However, an overlapping of the pathways may preclude this being an issue, especially with the NER pathway, which can correct damages that may normally be fixed by MMR proteins. The highest number of genes conserved in these pathways is in HR, with 81% of the genes present in both species. In humans, NER is the most important pathway for DNA repair. In elasmobranchs, MMR might be the most important pathway because all the significant genes are higher in the elephant shark than the human. NER is an especially versatile pathway and can repair any helix distorting damage, and we were able to find 70% of the 20 canonical human NER genes in the elephant shark, indicating these might be the only NER genes conserved.

Genes involved in the NER and BER pathways showed more genes that had significantly lower expression in elasmobranch than in humans. However, the HR and MMR pathways showed significantly increased expression of the genes in elasmobranchs than in humans. NHEJ had equal amount of lower and higher expression of the genes. Nurse sharks and yellow stingrays are both shallow-dwelling marine animals that receive high amounts of UV radiation, so it is interesting that they do not seem to have increased amounts of gene expression in the NER genes. However, this may be indictive of the shorter lifespan these animals have compared to that of humans. Furthermore, many of these genes are also used for replication, as well as overlapping with other DNA repair pathways. It is possible that NHEJ and MMR play larger roles in maintaining the integrity of the genome in these species. The genes not found in the elephant reference genome may be missing, yet to be discovered, have alternative names or aliases that are yet unknown. Some genes could be also found in the elephant shark genome but not in the human.

Of the five genes that are significantly higher in human compared to these two-elasmobranch species (Figure 10,11, and 12), the CSB and CSA genes are significantly higher in nurse shark compared to human. CSB and CSA genes are both involved in transcription-coupled repair in the human NER pathway, or the repair of actively transcribed genes. However, many of the global genomic repair genes are lower in

expression in elasmobranchs compared to that in human, a finding consistent with the higher NER expression in global genomic repair genes and longer lifespan in humans.

One of the limitations of this study is that there is not a lot of genomic information on elasmobranchs. The elephant shark genome has a simple genome and was the closest to our samples to be able to use as a reference genome for analysis of RNA sequencing data. All the elasmobranch samples were extracted on the dock of the NSU Oceanographic Center at relatively high temperatures, which might have affected the integrity of the tissue samples and thereby also the isolated RNA samples.

More studies must be done on a variety of elasmobranch species to get more data and to evaluate the overall trends in gene expression. In particular, elasmobranch species with different lifespans should be obtained and their RNA sequenced. A much more complex study is to run a DNA copy number analysis to see how many copies of each genes is present in each individual, there might be lesser or fewer copies of the genes in question. Lastly, the nurse shark and yellow stingray samples have to be performed by *de novo* transcriptome assembly, to construct a transcriptome for this species to obtain more accurate results for elasmobranch species.

Conclusion

Elasmobranchs are many million years apart from humans phylogenetically. The main goal for this project was to discover more about the genomic information of elasmobranchs, comparing the human genes with the elephant shark genes. In addition, two local elasmobranch species provided information on DNA repair pathways and respective lifespans. The correlation of DNA repair with a longer lifespan was shown in this project with the nucleotide excision repair (NER) pathway. The NER pathway is apparently an evolutionarily important mechanism, as evidenced by our finding of 14 out of 20 human NER genes shared as orthologs in elasmobranchs, regardless of the 400-million-year evolutionary difference between the taxa.

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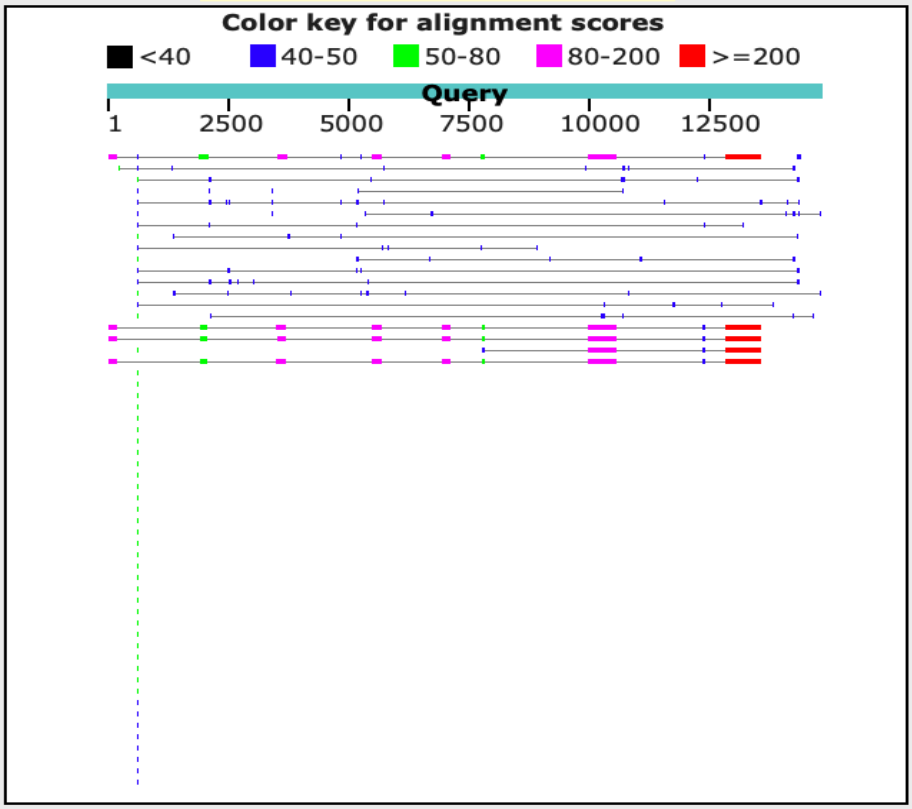
Appendix

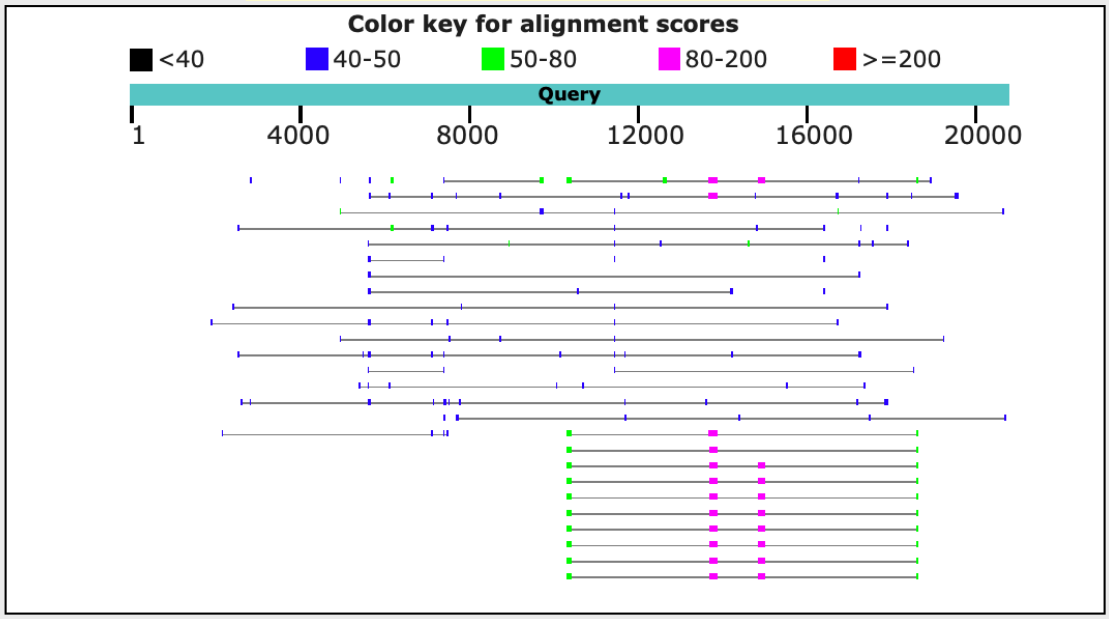
XPF Distribution of the top 219 Blast Hits on 100 subject sequences **73%**

Mouse over to see the title, click to show alignments



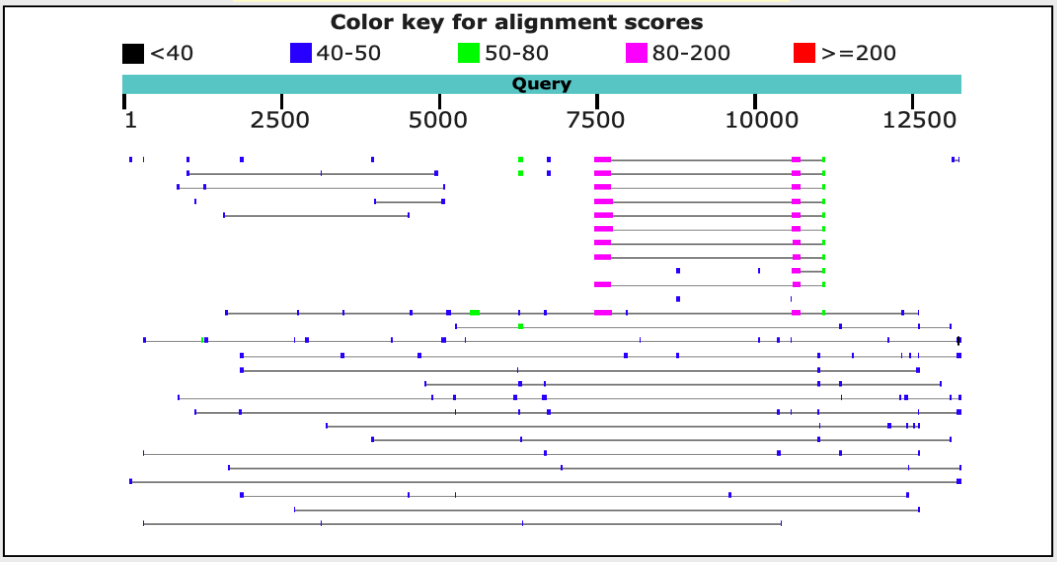
CCNH Distribution of the top 170 Blast Hits on 39 subject sequences **77%**

Mouse over to see the title, click to show alignments



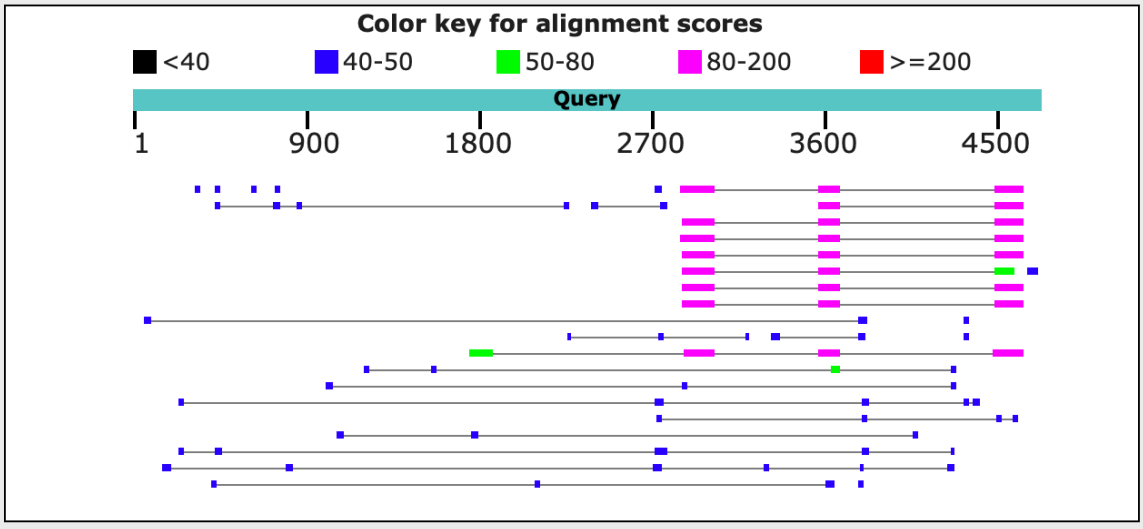
XPC Distribution of the top 169 Blast Hits on 45 subject sequences **76%**

Mouse over to see the title, click to show alignments



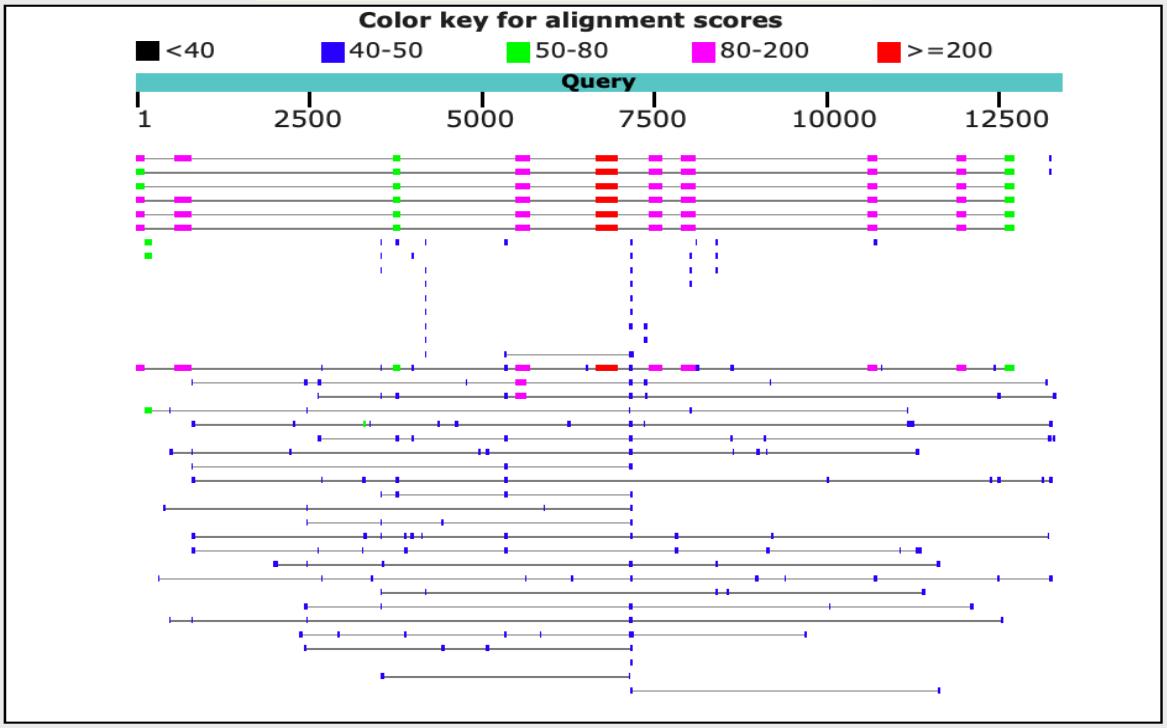
XPA Distribution of the top 84 Blast Hits on 31 subject sequences **81%**

Mouse over to see the title, click to show alignments



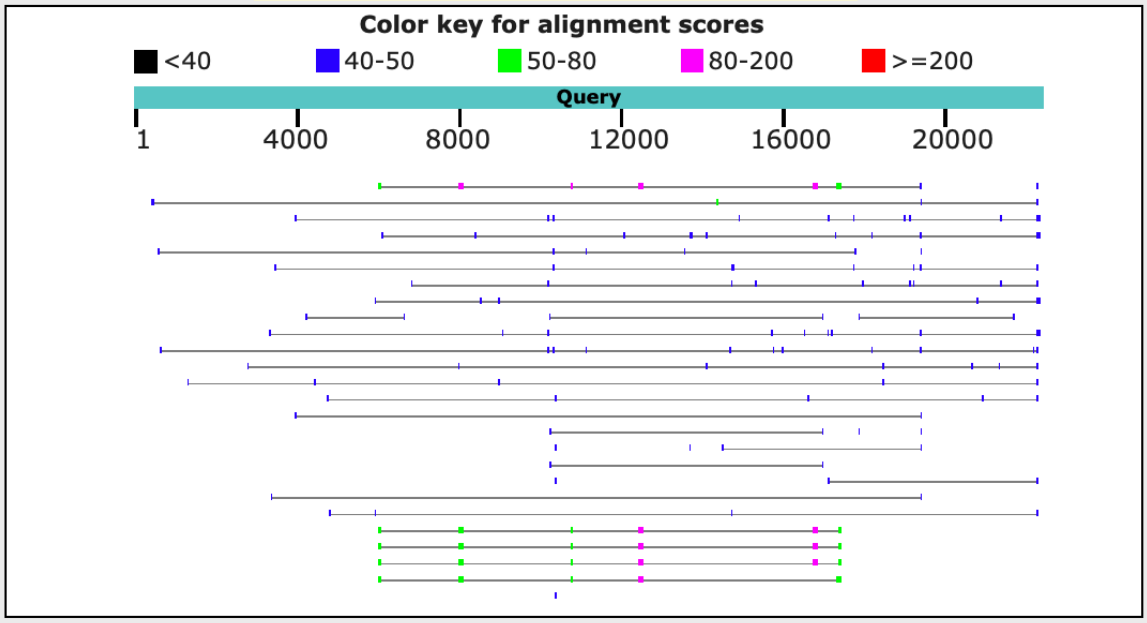
XPB Distribution of the top 285 Blast Hits on 66 subject sequences **81%**

Mouse over to see the title, click to show alignments



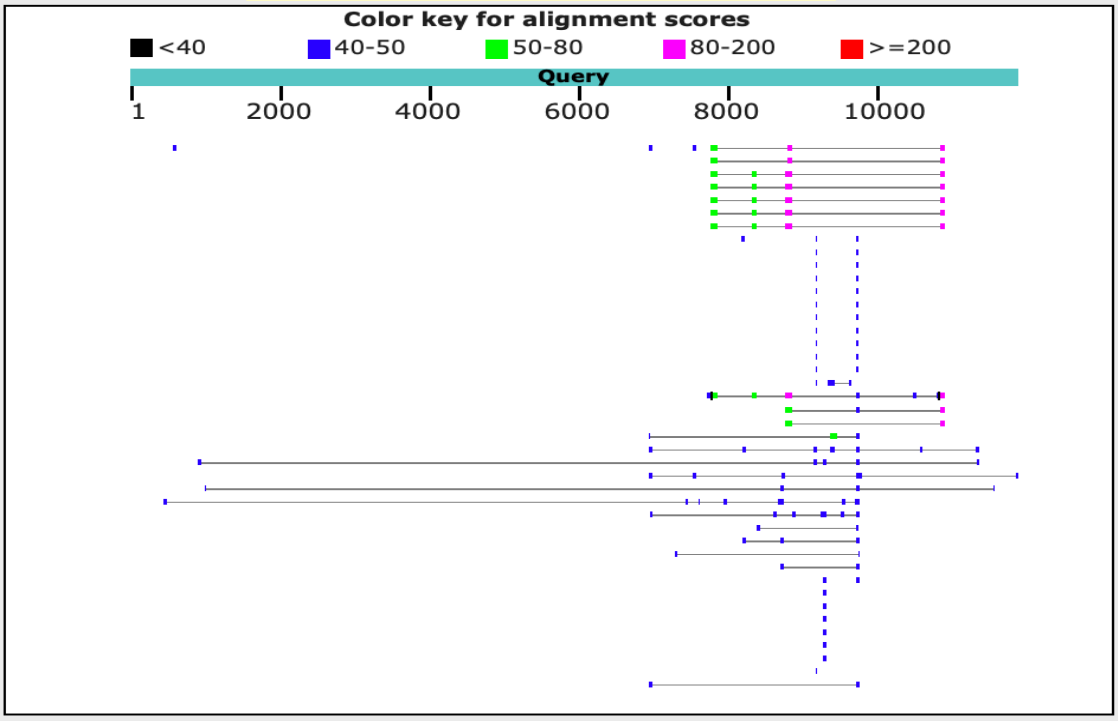
RPA1 Distribution of the top 162 Blast Hits on 35 subject sequences **75%**

Mouse over to see the title, click to show alignments



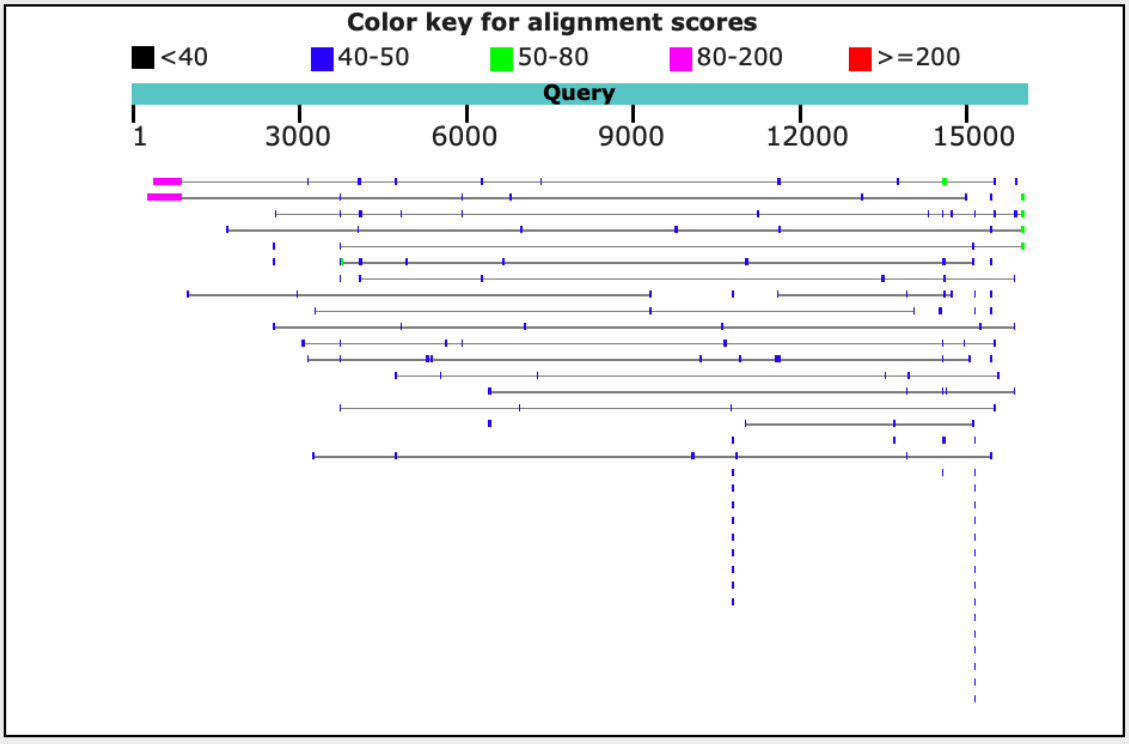
RPA2 Distribution of the top 161 Blast Hits on 59 subject sequences **86%**

Mouse over to see the title, click to show alignments



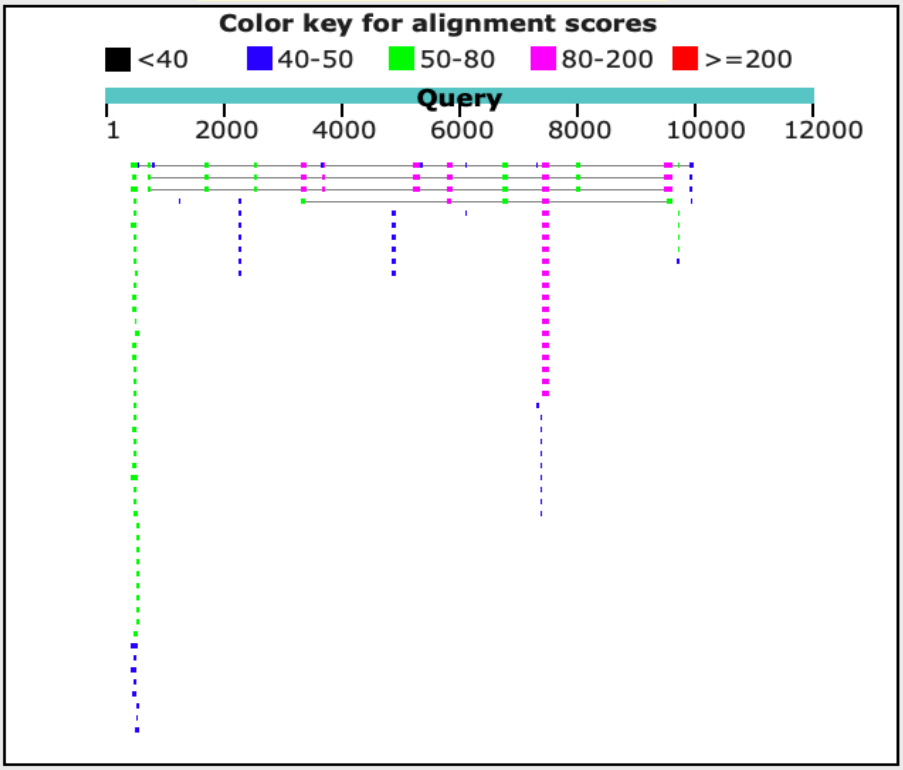
RPA3 Distribution of the top 164 Blast Hits on 62 subject sequences **72%**

Mouse over to see the title, click to show alignments



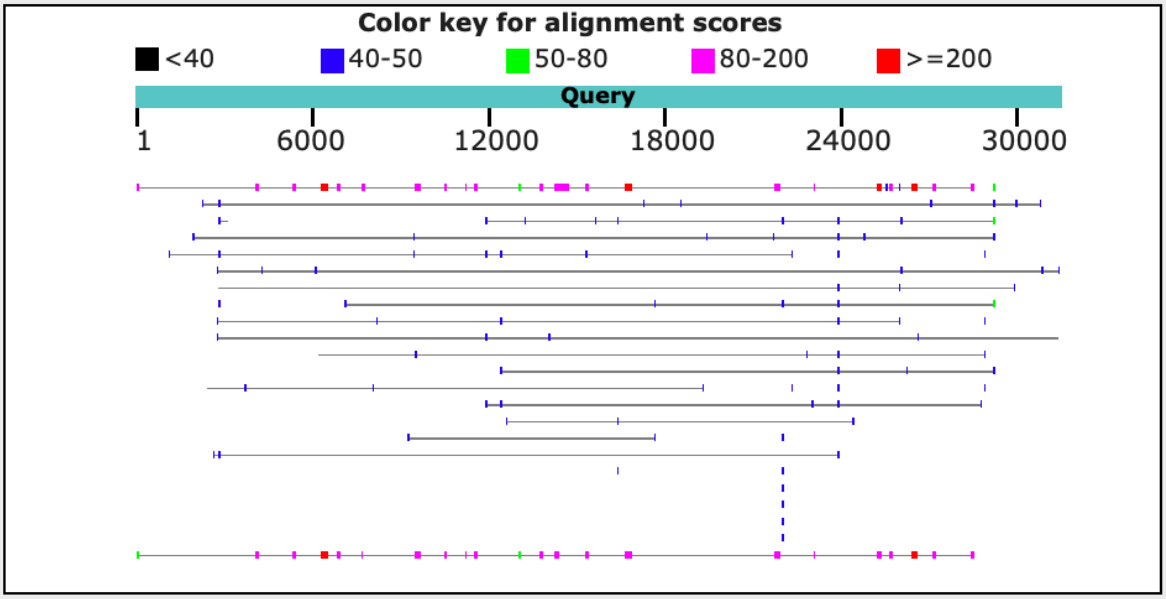
GTF2H2 Distribution of the top 200 Blast Hits on 100 subject sequences **82%**

Mouse over to see the title, click to show alignments



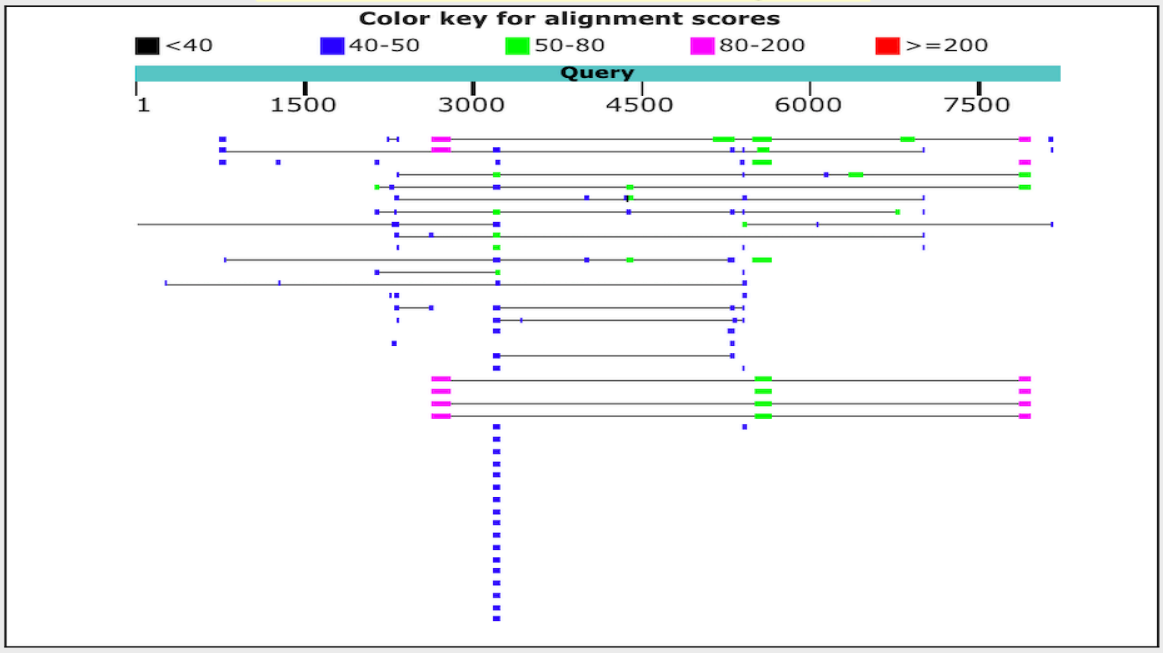
DDB1 Distribution of the top 164 Blast Hits on 35 subject sequences **80%**

Mouse over to see the title, click to show alignments



CSB Distribution of the top 164 Blast Hits on 58 subject sequences **74%**

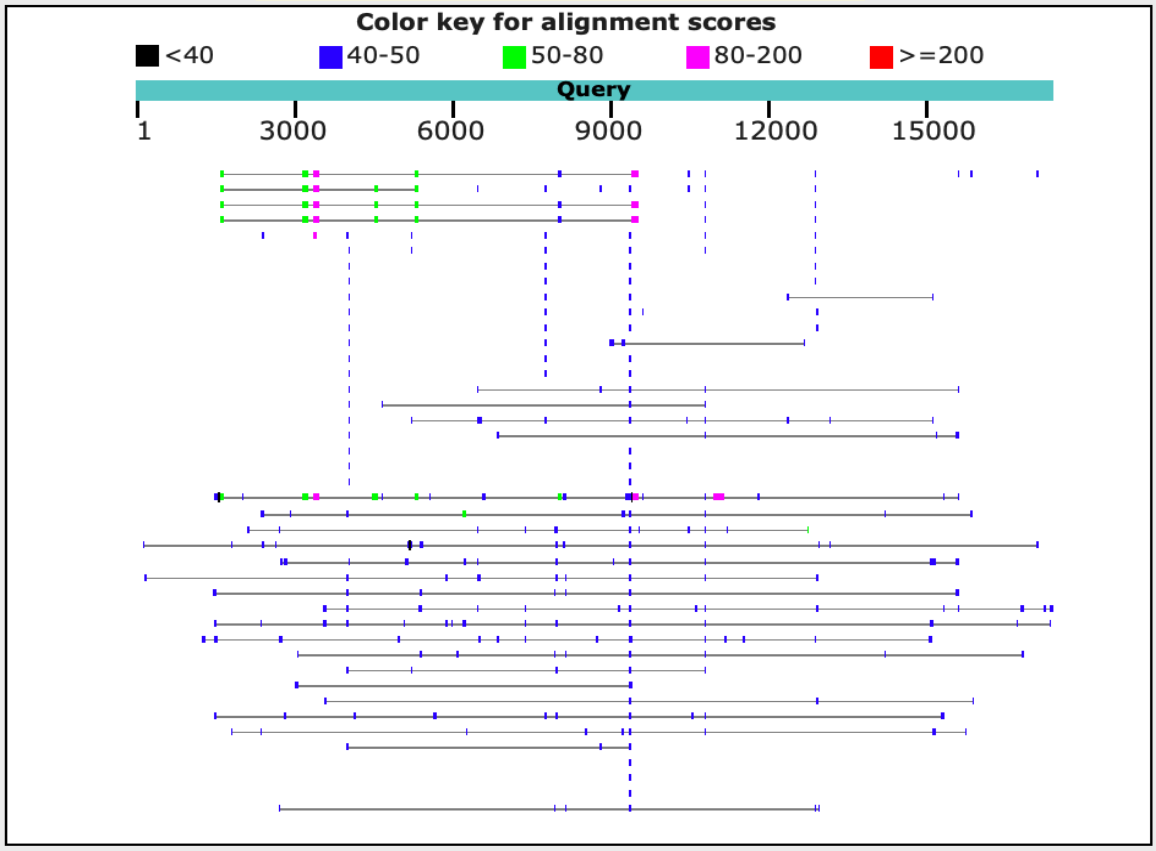
Mouse over to see the title, click to show alignments



Graphic Summary

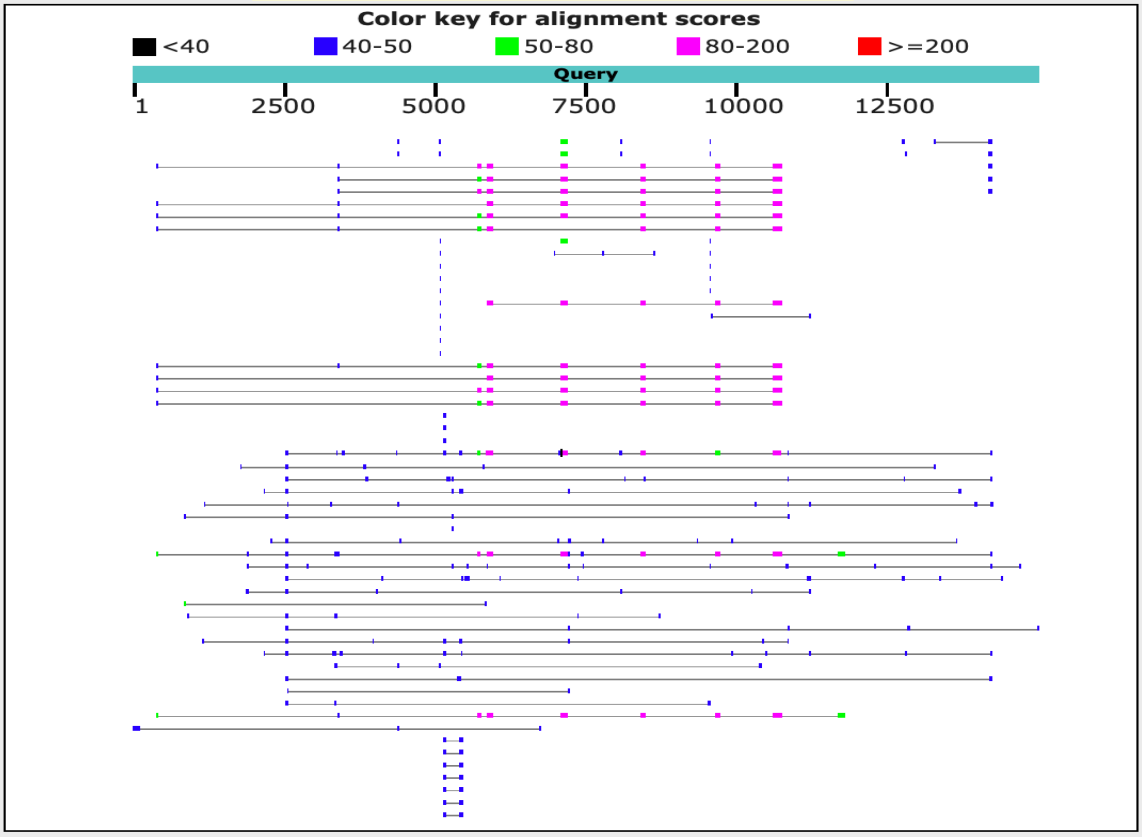
CSA Distribution of the top 392 Blast Hits on 100 subject sequences **87%**

Mouse over to see the title, click to show alignments



CDK7 Distribution of the top 362 Blast Hits on 79 subject sequences 89%

Mouse over to see the title, click to show alignments



GTF2H3 Distribution of the top 99 Blast Hits on 40 subject sequences **67%**

Mouse over to see the title, click to show alignments